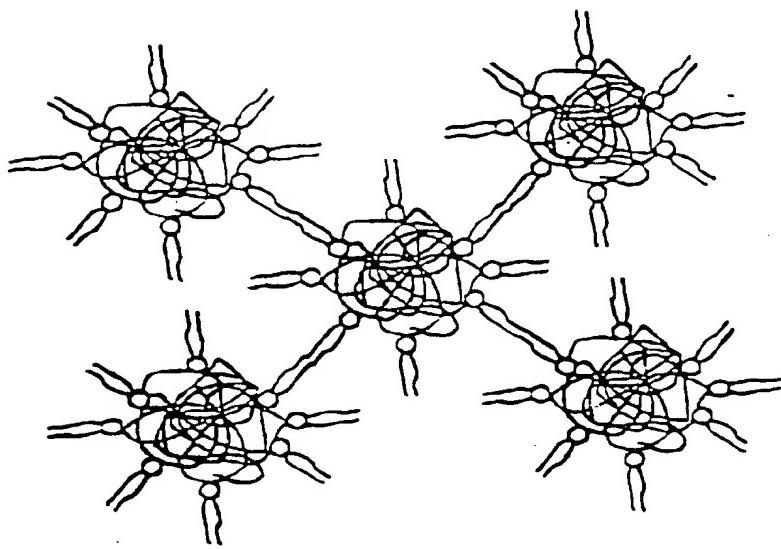


Figure 1

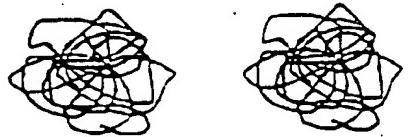
Sticky DNA



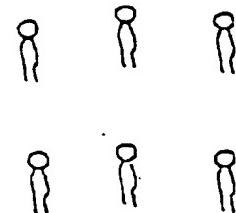
**aggregation
precipitation**

Figure 2

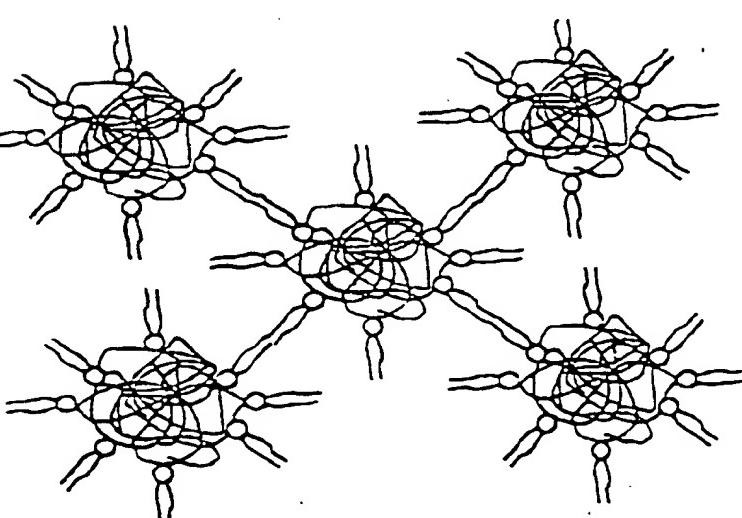
Negatively Charged Plasma



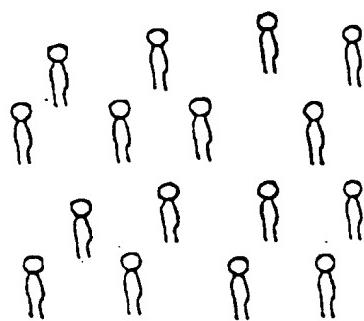
Positively Charged Lipid



Sticky DNA



Excess Lipids



Plasmid Encapsulated Within a Lipid Bilayer

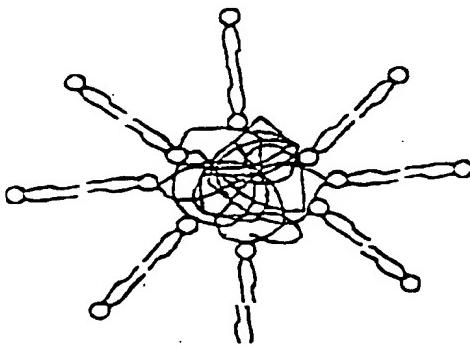
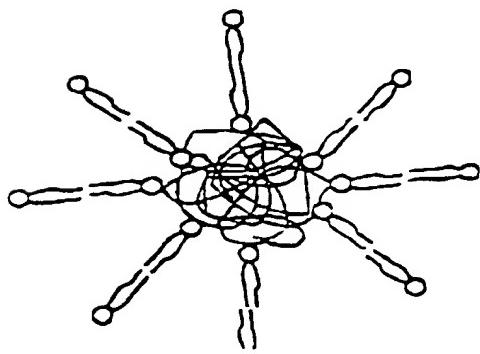


Figure 3

05/31/96 02:32

604 264 9959

INEX

025/043

Figure 1
Recovery of DNA After Extrusion
(20 mg total lipid)

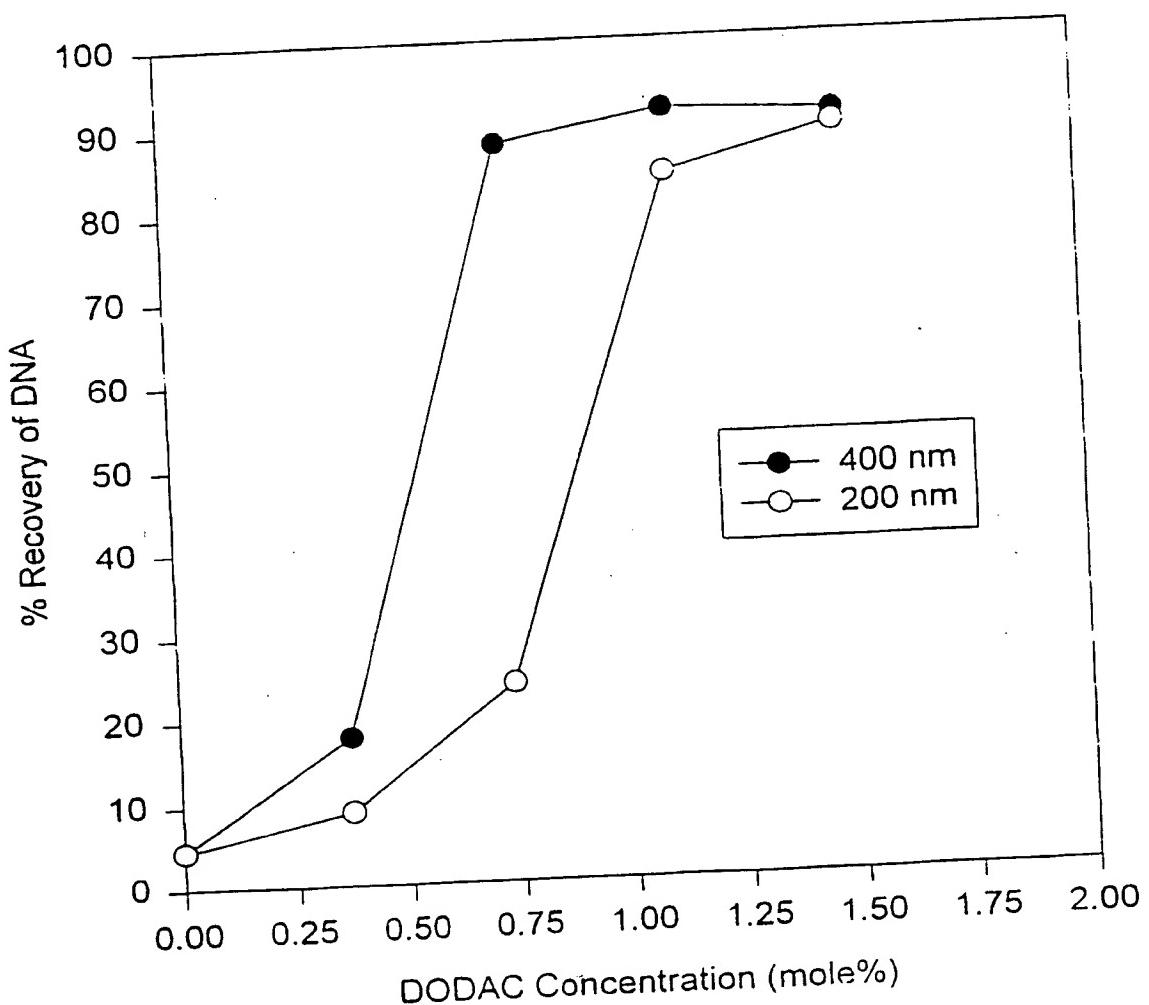


Figure 4

05/31/96 02:33

604 264 9959

INEX

026/043

Figure 2
Anion Exchange Chromatography
(20 mg total lipid)

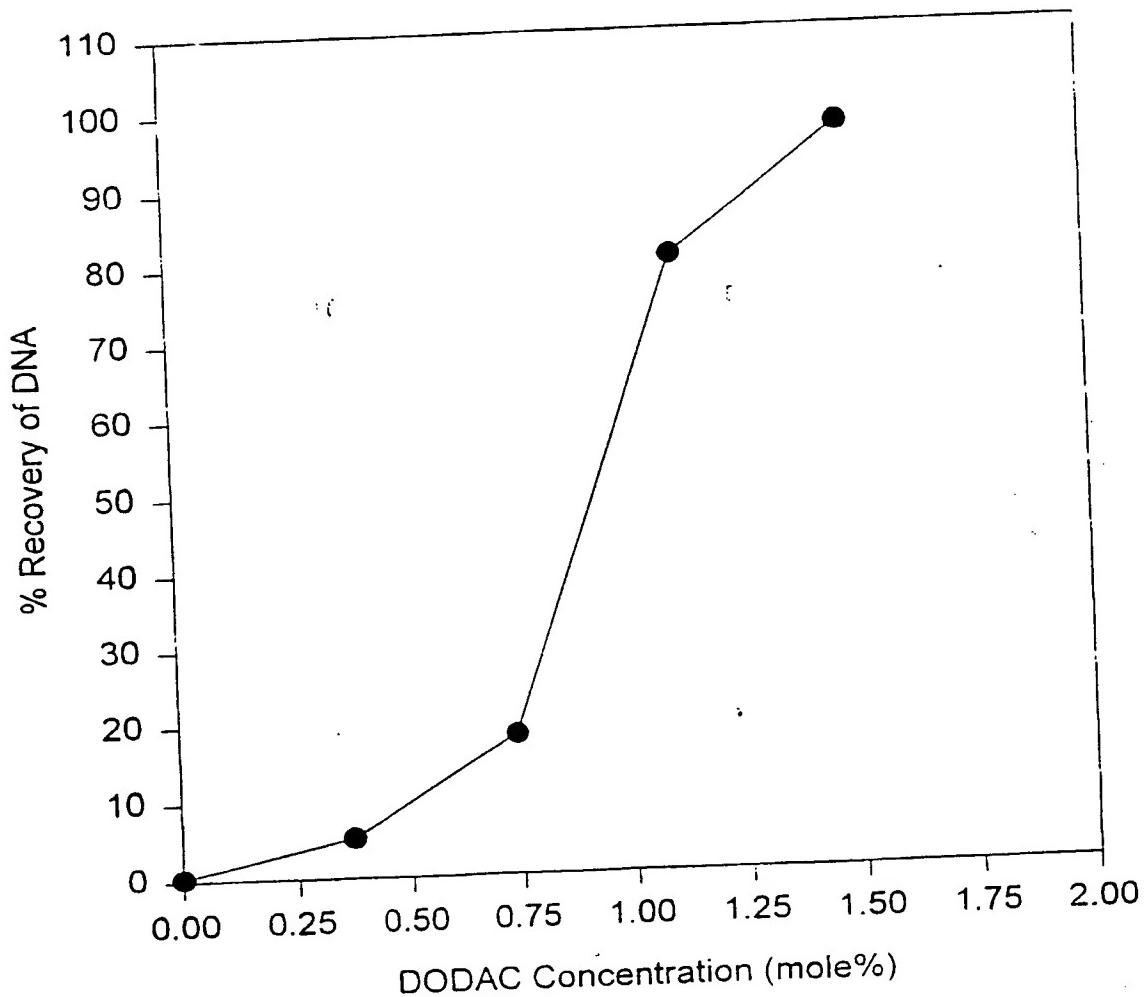


Figure 5

05/31/96

02:33

604 264 9959

INEX

027/043

Recovery of Lipid After Extrusion
POPC:DODAC:PEG-Cer(C20), 20 mg

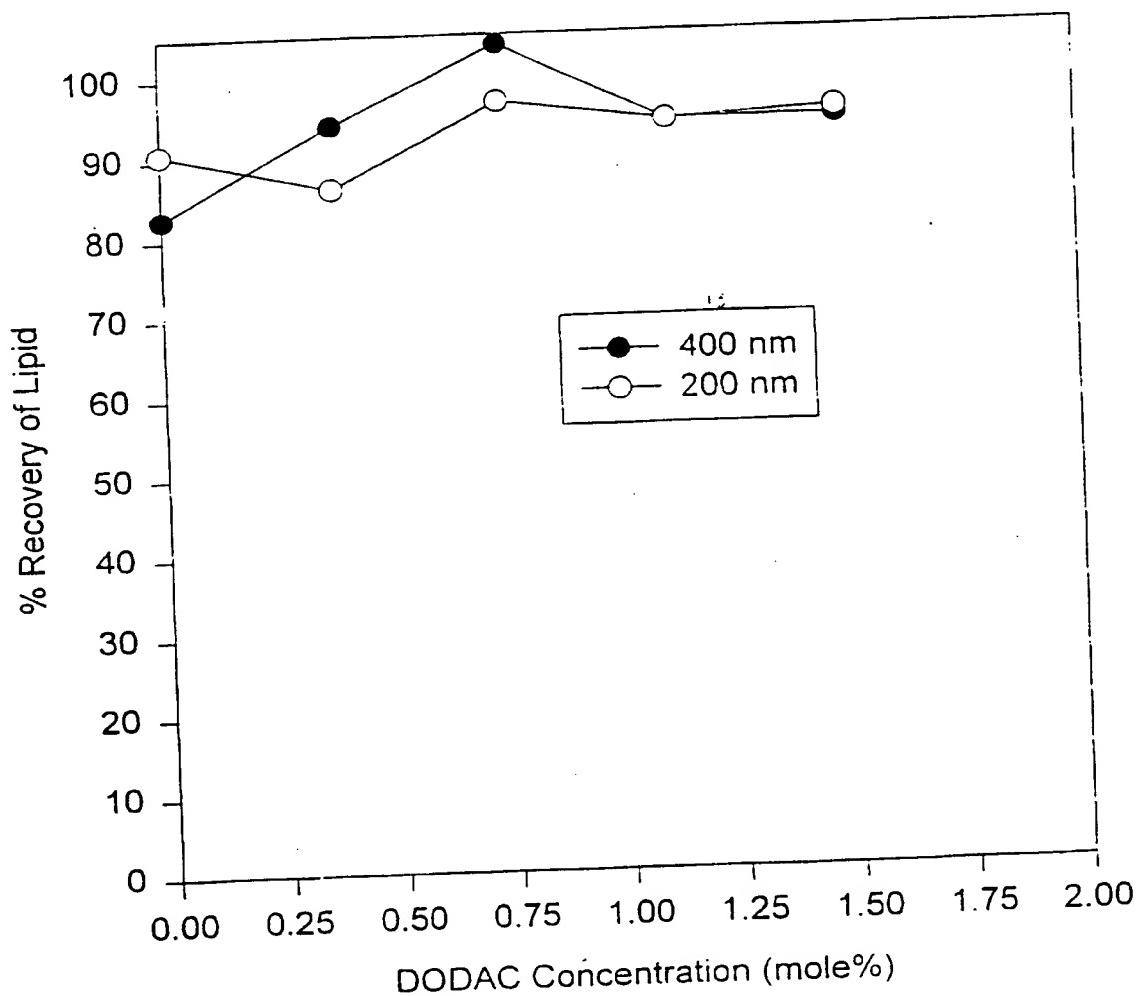


Figure 6

05/31/96 02:34

604 264 9959

INEX

028/043

Anion Exchange Chromatography
POPC:DODAC:PEG-Cer(C20), 20 mg

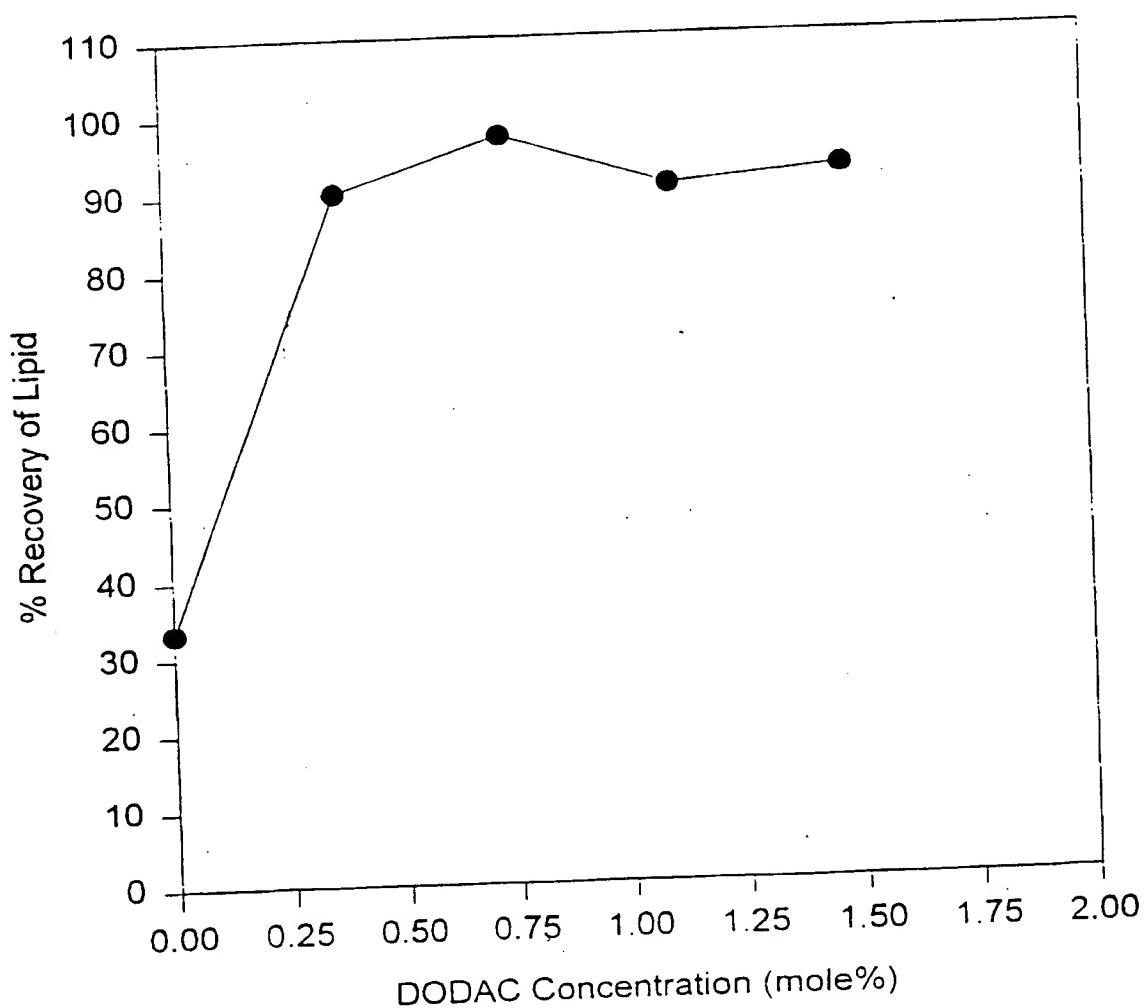


Figure 7

05/31/96 02:34

604 264 9959

INEX

029/043

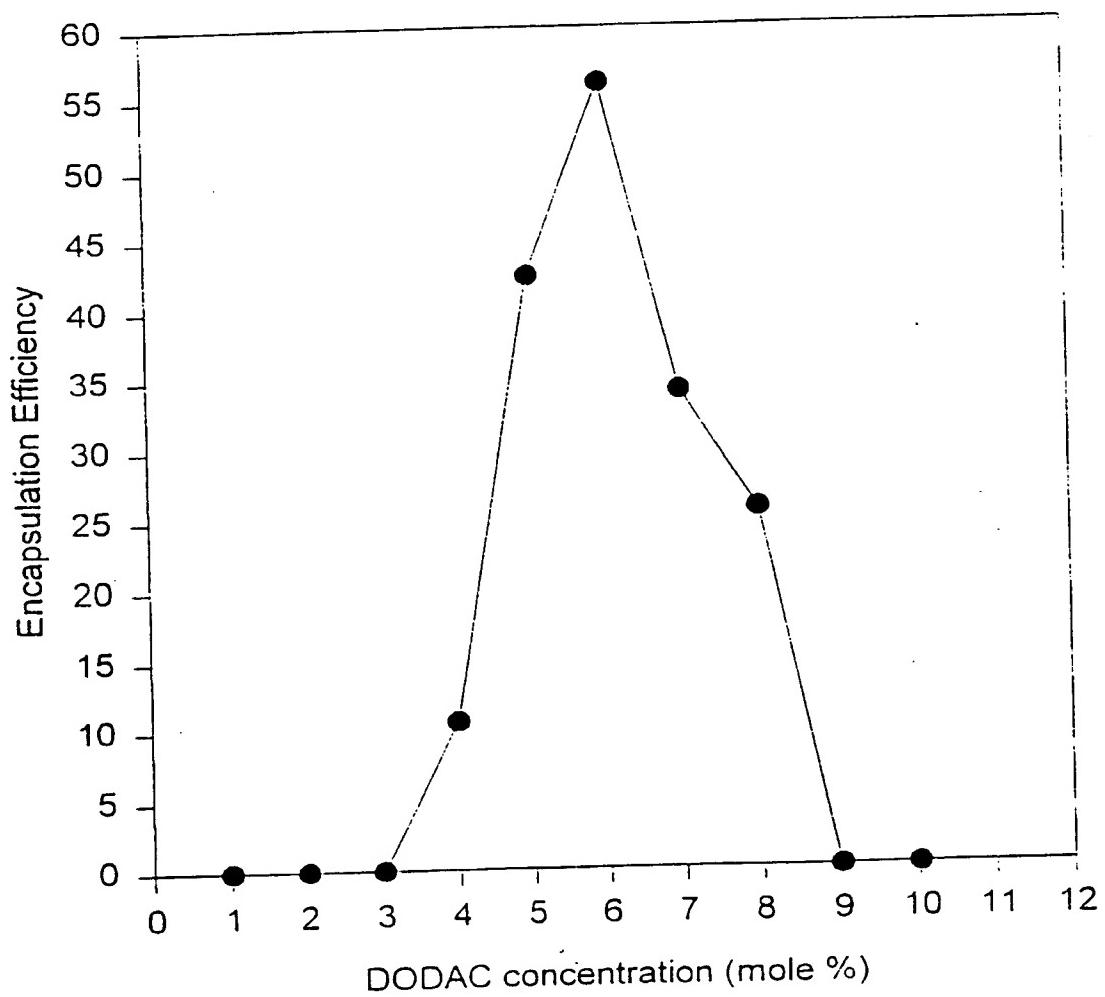


Figure 8

05/31/96

02:34

604 264 9959

INEX

030/043

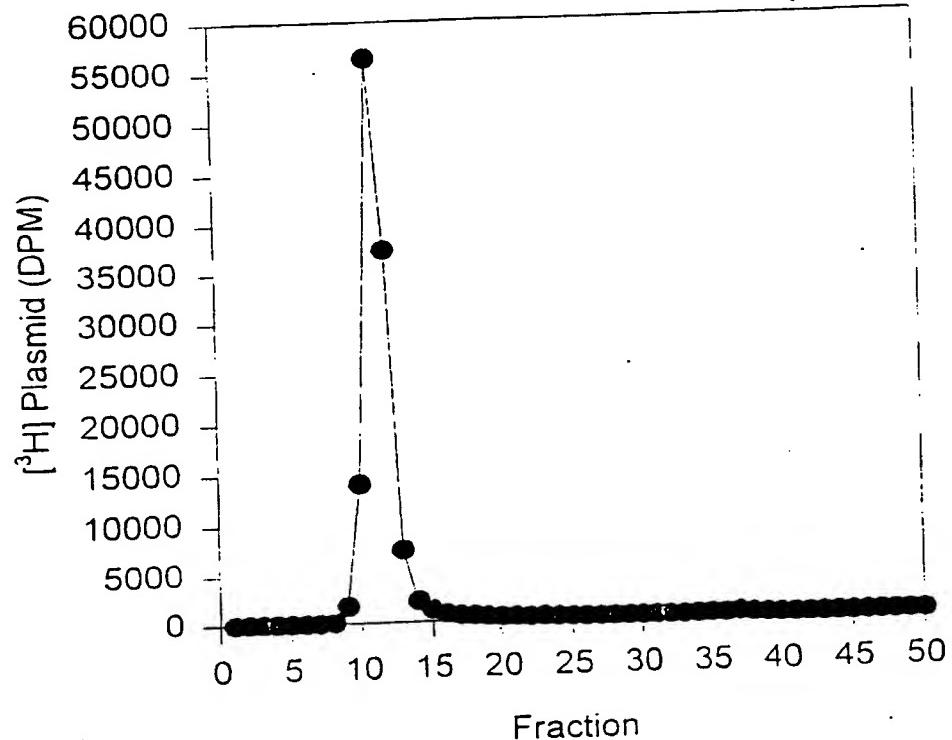


Figure 9A

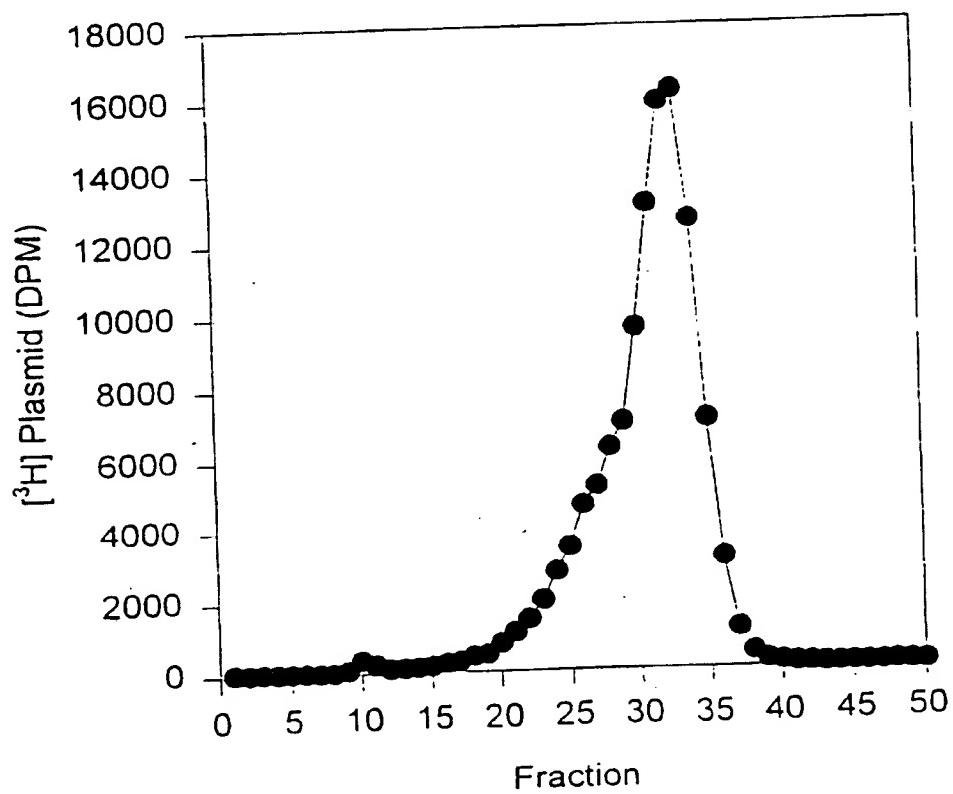


Figure 9B

05/31/96 02:35

604 264 9959

INEX

031/043

Recovery of ^3H DNA and ^{14}C Lipid
After Incubation in Mouse Serum
POPC:DODAC:PEG-Cer(C20)

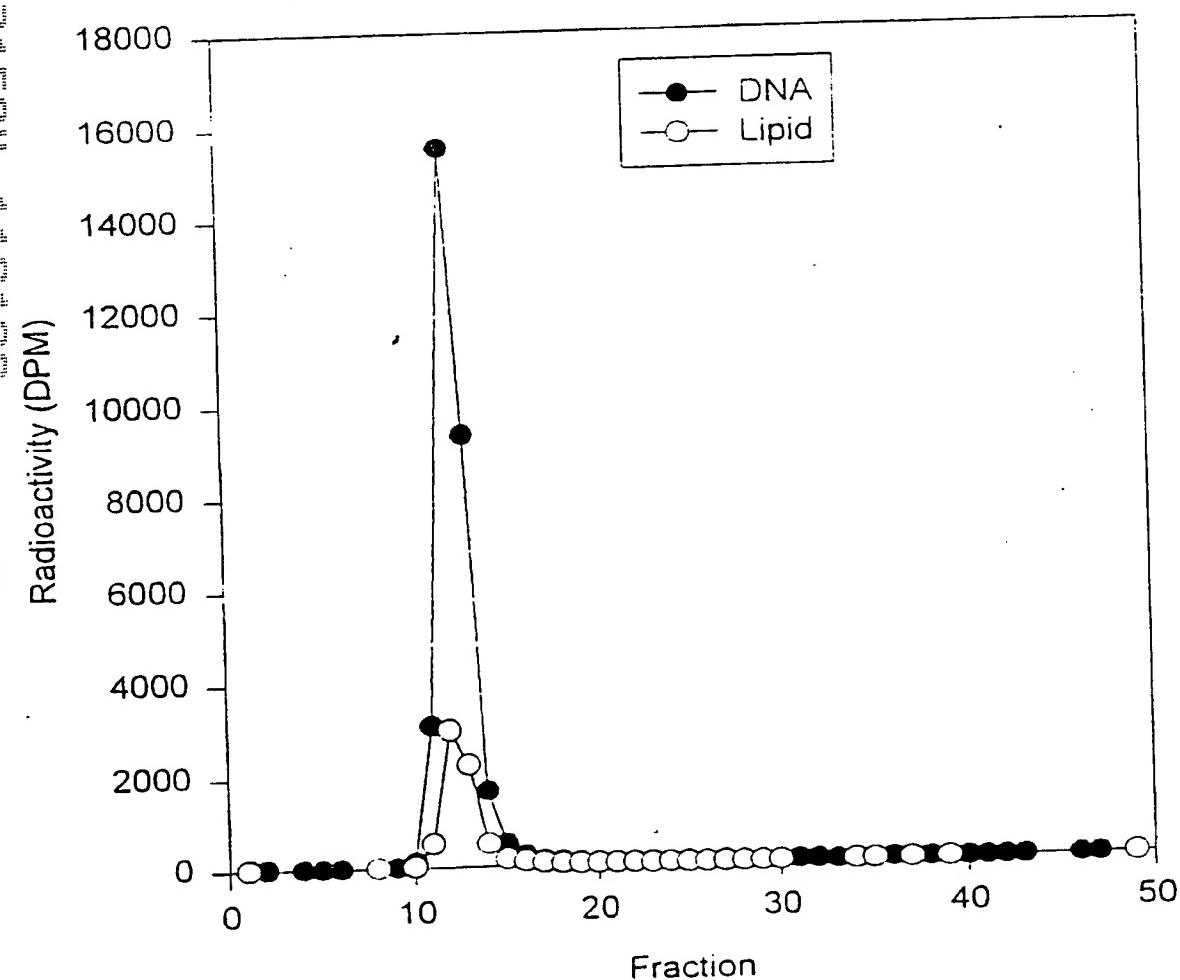


Figure 10

Figure 11A

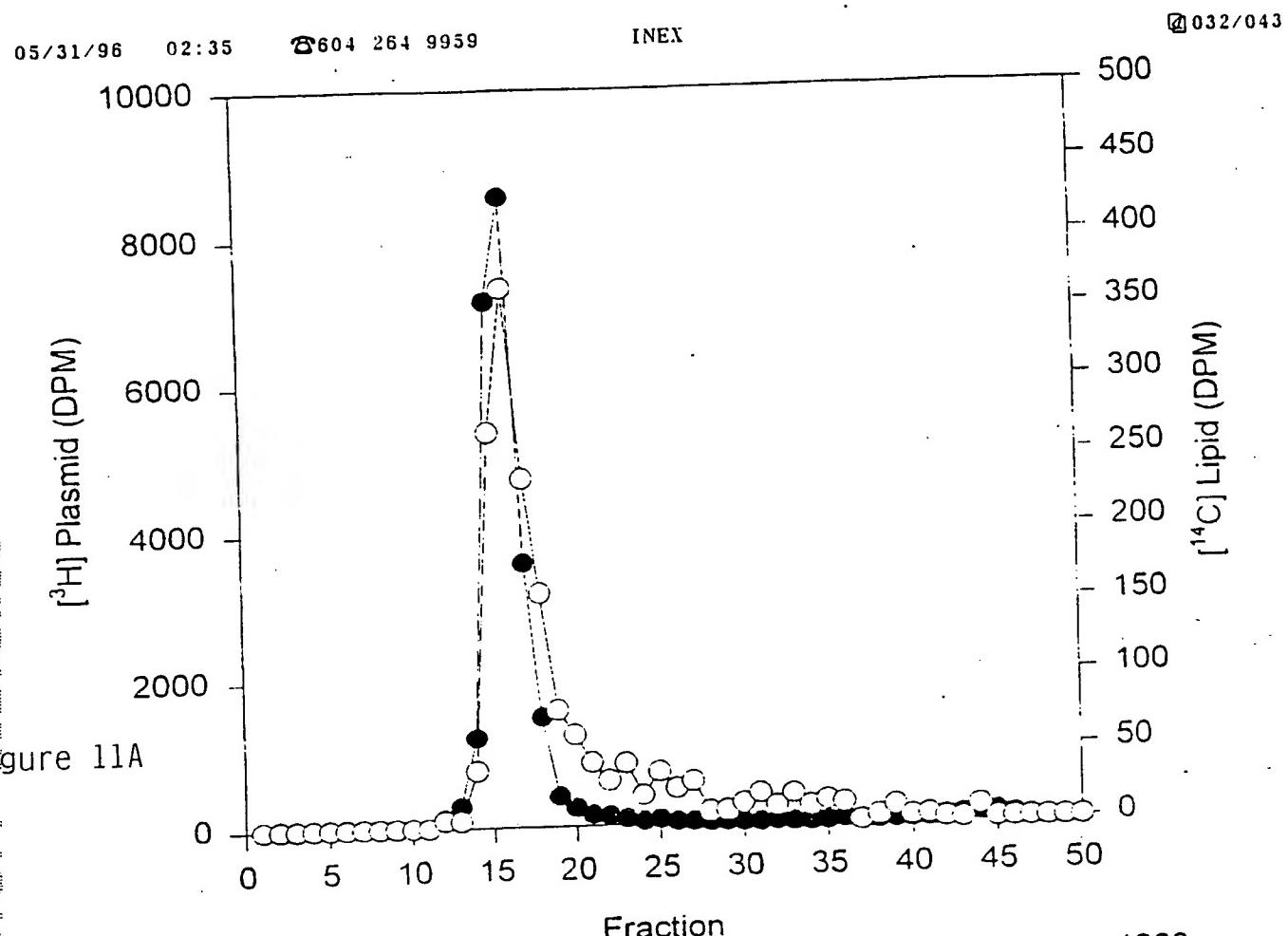
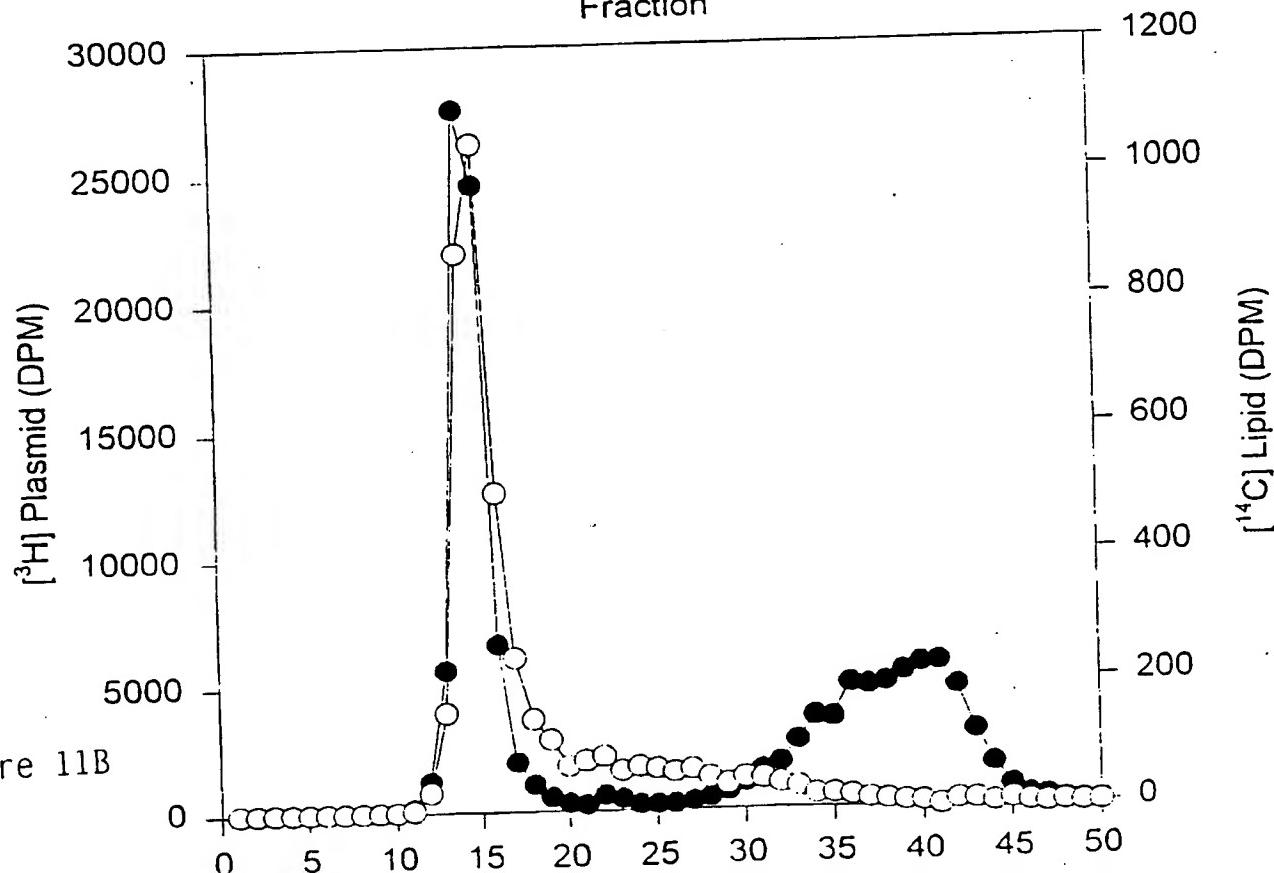


Figure 11B



05/31/96 02:36

604 264 9959

INEX

033/043

A

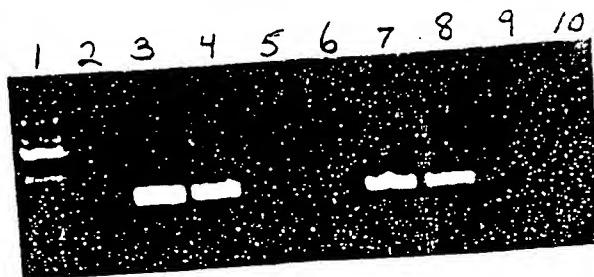


Figure 12A

B

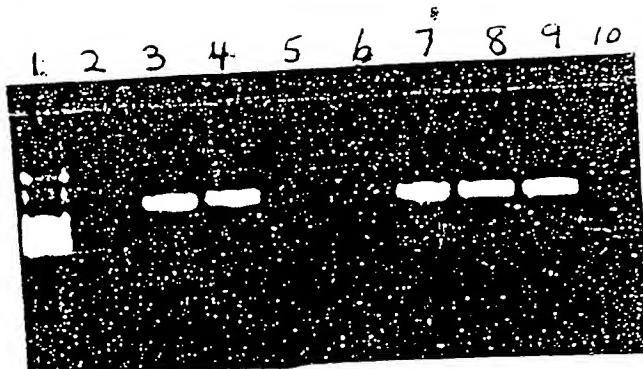


Figure 12B

05/31/96

02:37

604 264 9959

INEX

034/043

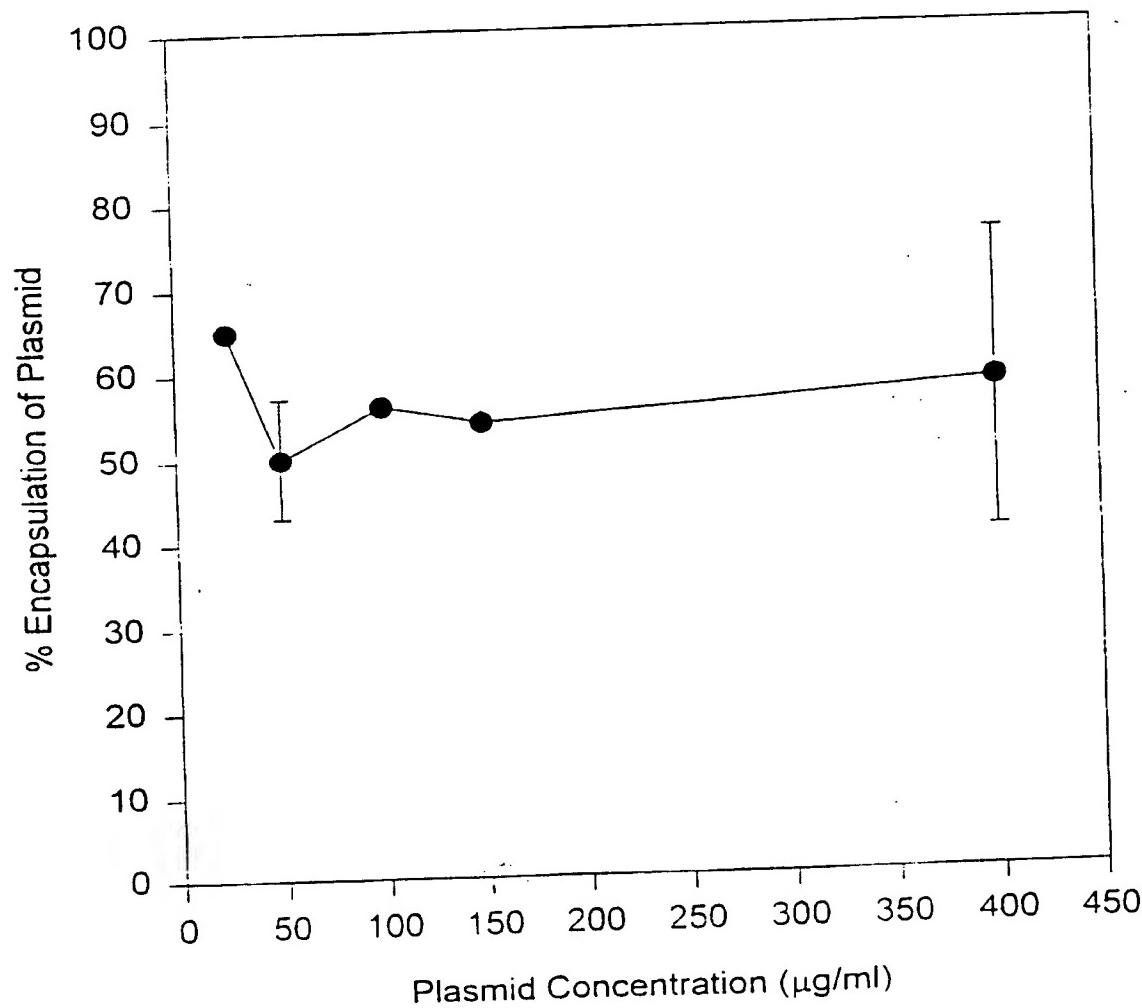


Figure 13

05/31/96 02:37

604 264 9959

INEX

035/043

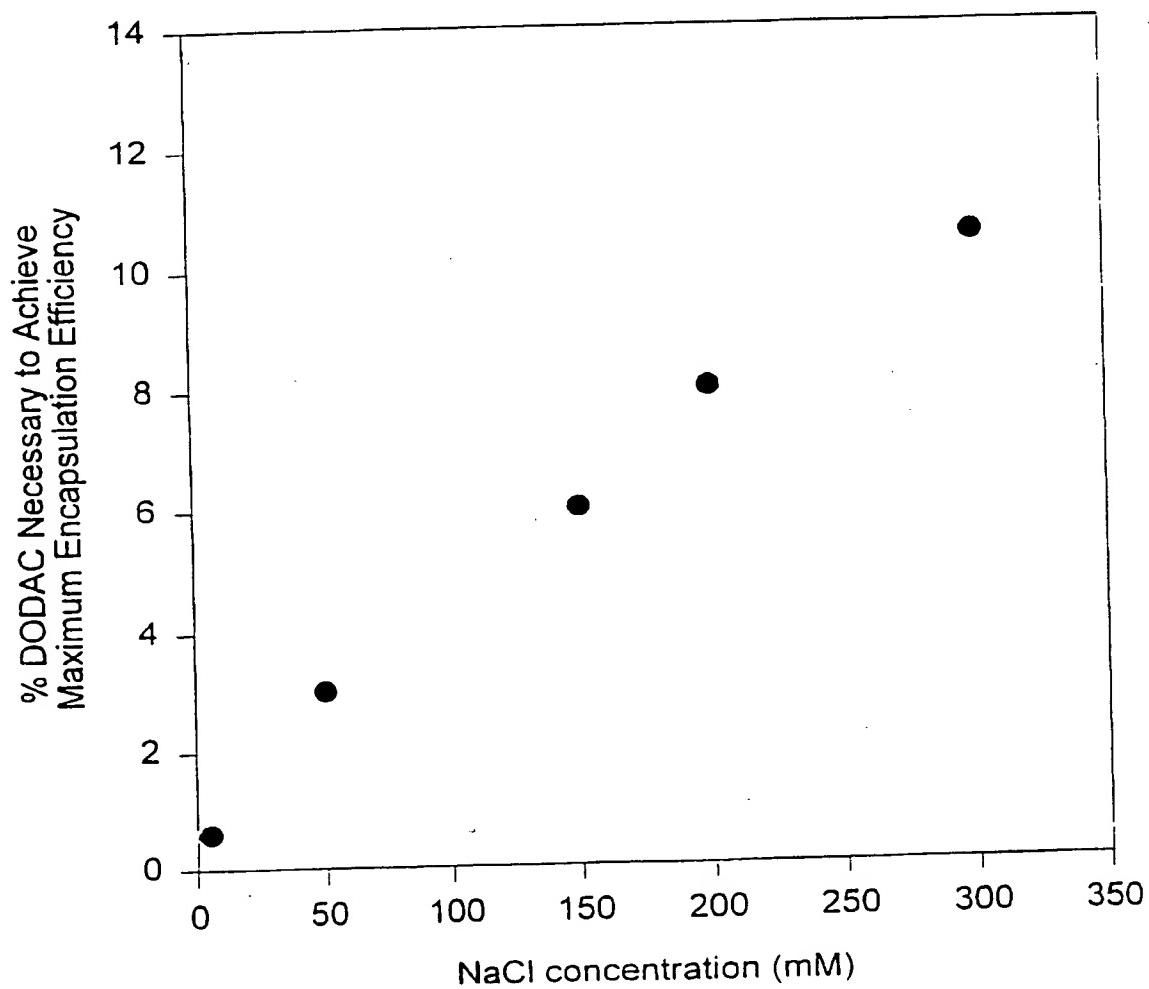


Figure 14

05/31/96 02:37 604 264 9959

INEX

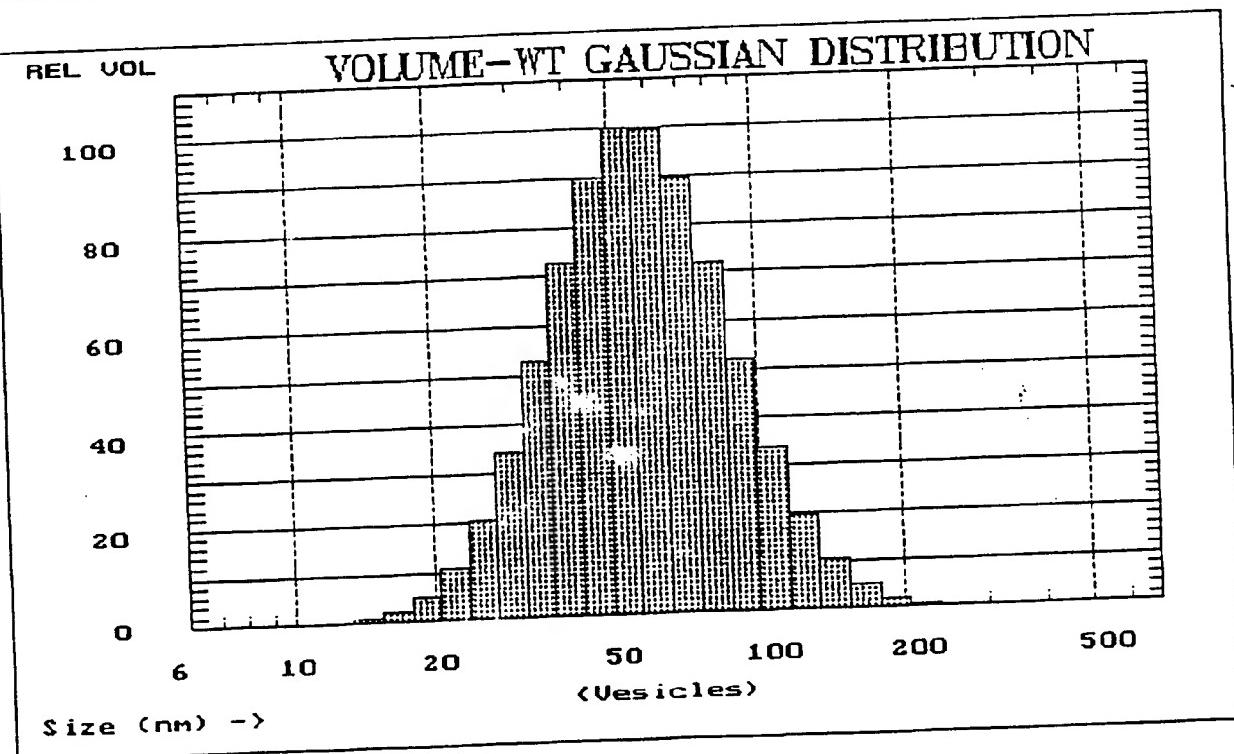
036/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment
Stored Data File a:\PEGC20.06

VOLUME-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 61.6 nm Chi Squared = 0.347
Stnd. Deviation = 27.0 nm (43.9 %) Baseline Adj. = 0.000 %
Coeff. of Var'n = 0.439 Mean Diff. Coeff. = 7.54E-08 cm²/s



Cumulative Results:

25 % of distribution < 38.73 nm
50 % of distribution < 52.05 nm
75 % of distribution < 69.91 nm
90 % of distribution < 91.36 nm
99 % of distribution < 142.99 nm

Figure 15

Run Time = 1 Hr 43 Min 26 Sec
Count Rate = 303 KHz
Channel #1 = 2827.4 K
Channel Width = 8.0 uSec

Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

05/31/96 02:38 604 264 9959

INEX

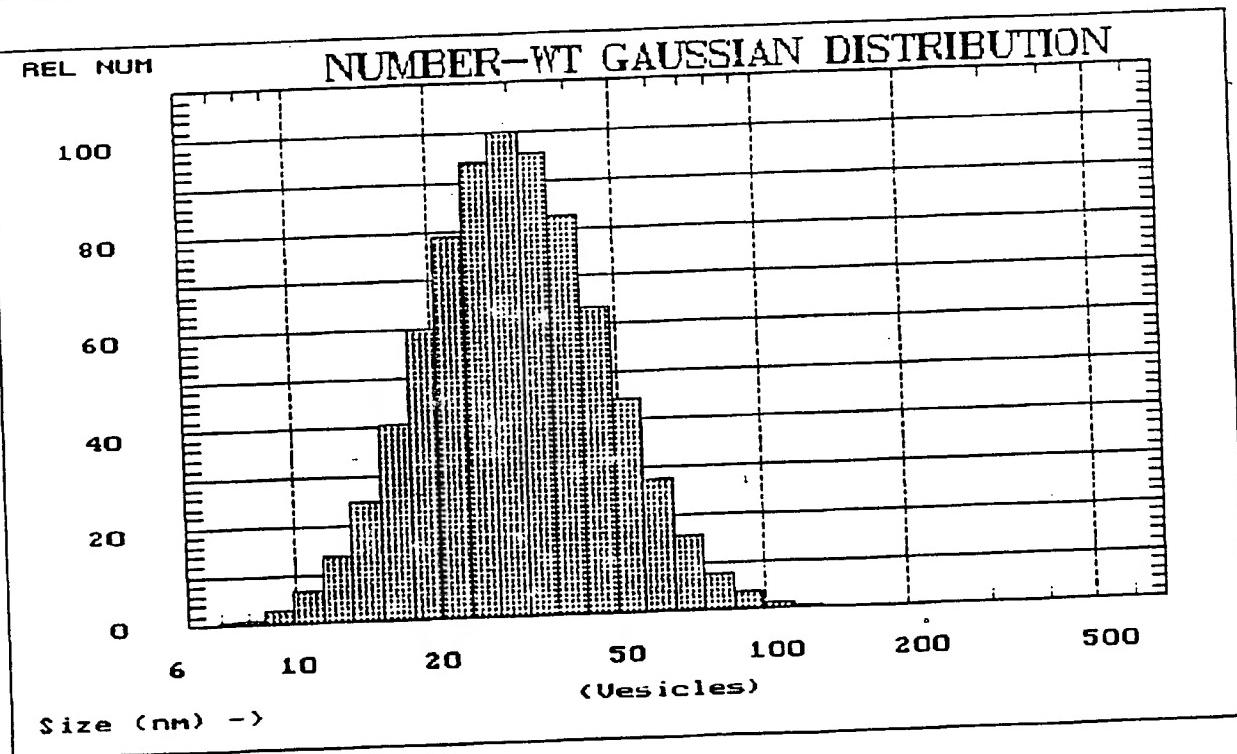
037/043

DOPE:DODAC:PEG-Cer(C20) (84:6:10) for animal experiment
Stored Data File a:\PEGC20.06

NUMBER-Weighted GAUSSIAN Analysis (Vesicles)

GAUSSIAN SUMMARY:

Mean Diameter = 32.8 nm Chi Squared = 0.347
Stnd. Deviation = 14.4 nm (43.9 %) Baseline Adj. = 0.000 %
Coeff. of Var'n = 0.439 Mean Diff. Coeff. = 1.42E-07 cm²/s



Cumulative Results:

25 % of distribution < 20.56 nm
50 % of distribution < 27.72 nm
75 % of distribution < 37.35 nm
90 % of distribution < 48.88 nm
99 % of distribution < 77.28 nm

Figure 16

Run Time = 1 Hr 43 Min 26 Sec
Count Rate = 303 KHz
Channel #1 = 2827.4 K
Channel Width = 8.0 uSec

Wavelength = 632.8 nm
Temperature = 23 deg C
Viscosity = 0.933 cp
Index of Ref. = 1.333

PLASMID TGS

Figure 17A

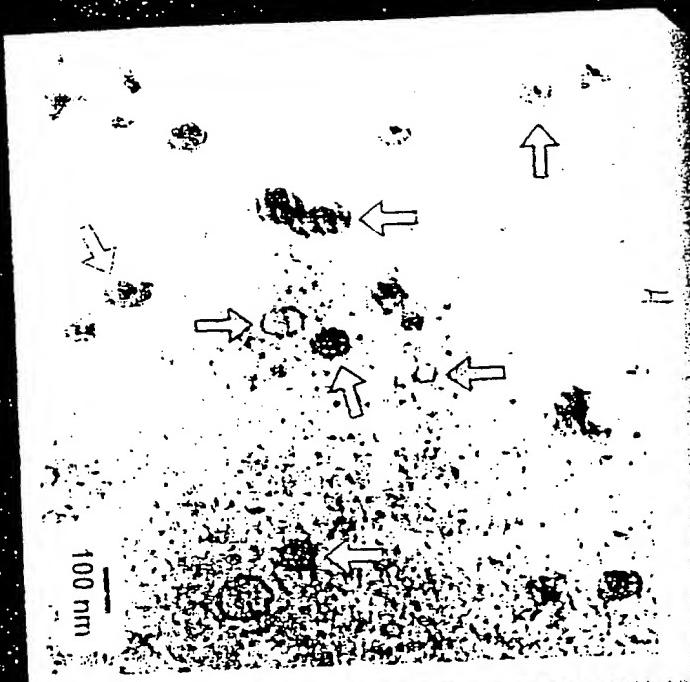
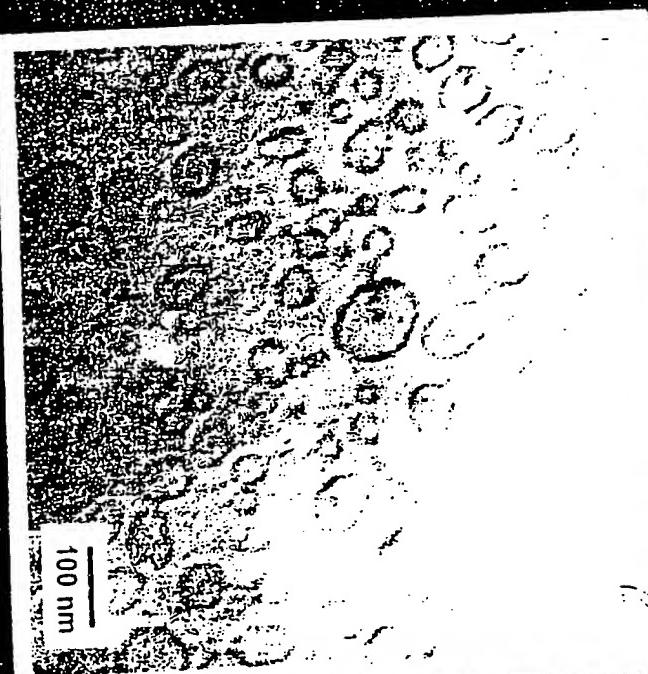


Figure 17B



INEX

05/31/96

02:41

604 264 9959

INEX

039/043

Clearance of DNA Encapsulated in POPC:DODAC:PEG-Cer(C20)

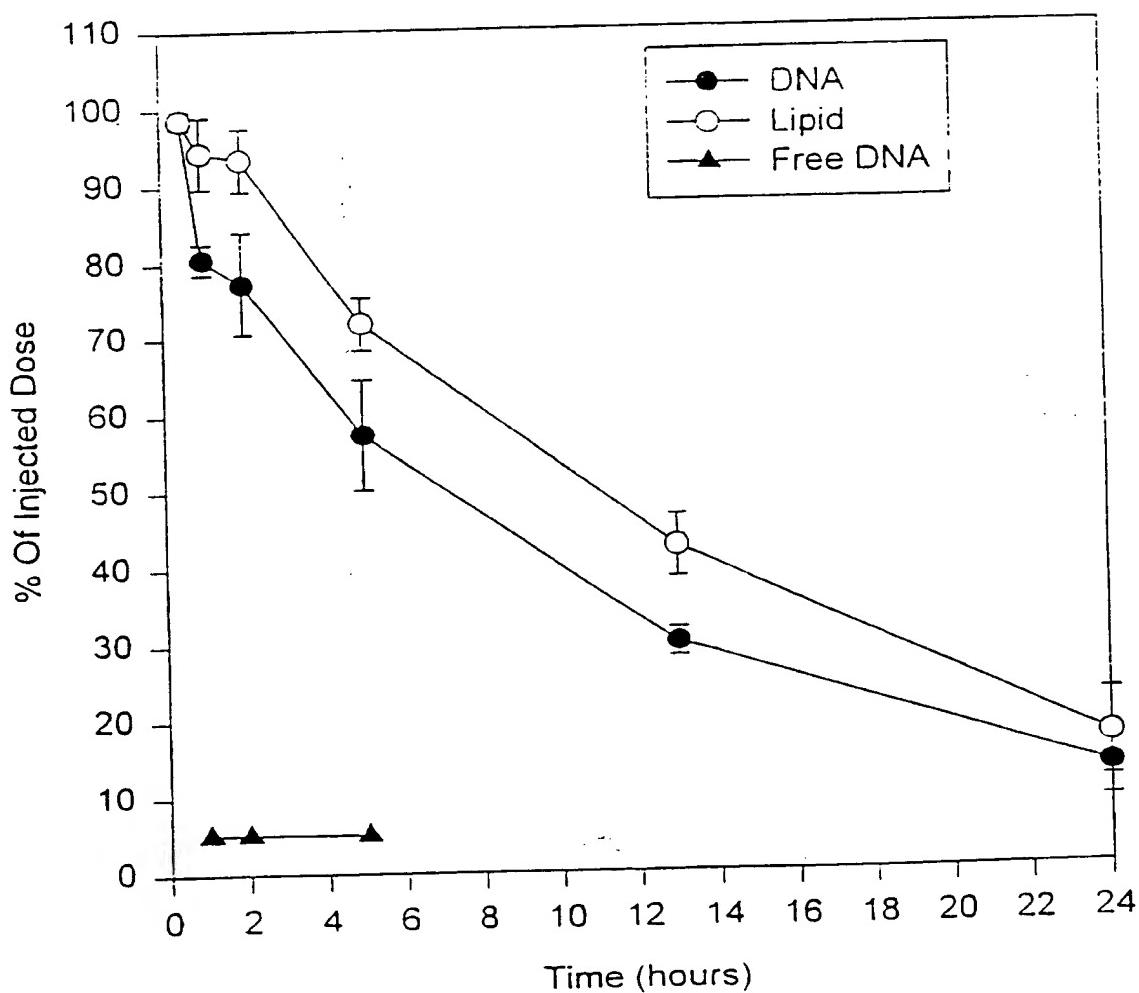


Figure 18

05/31/96

02:42

604 264 9959

INEX

040/043

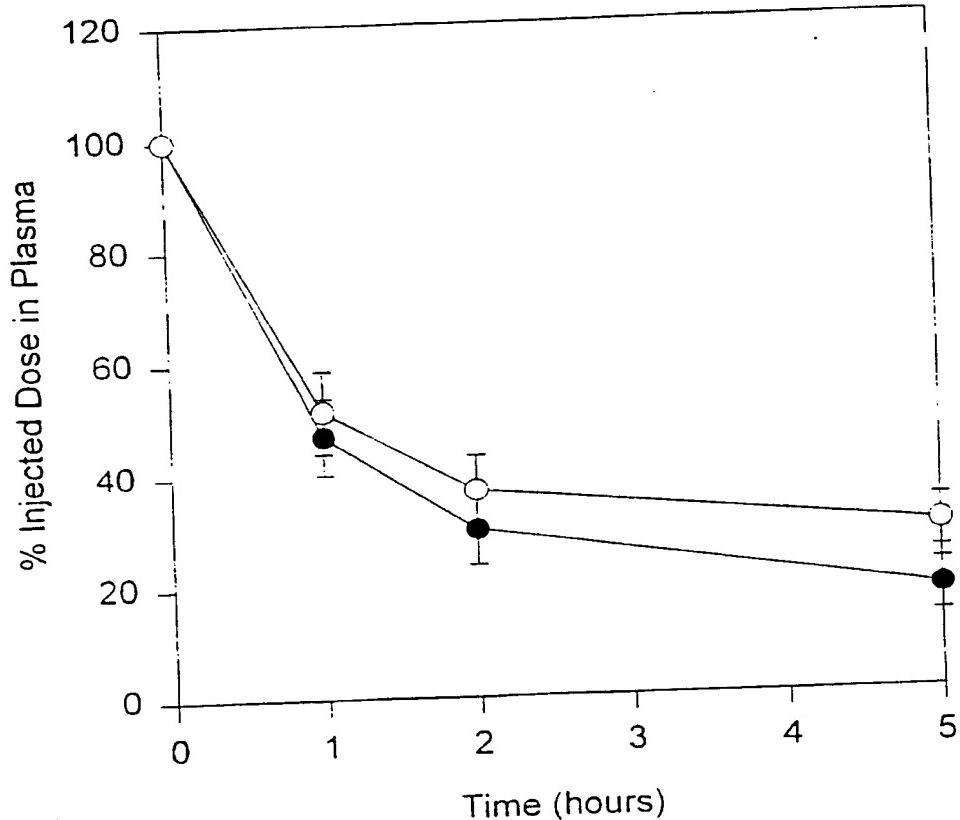


Figure 19A

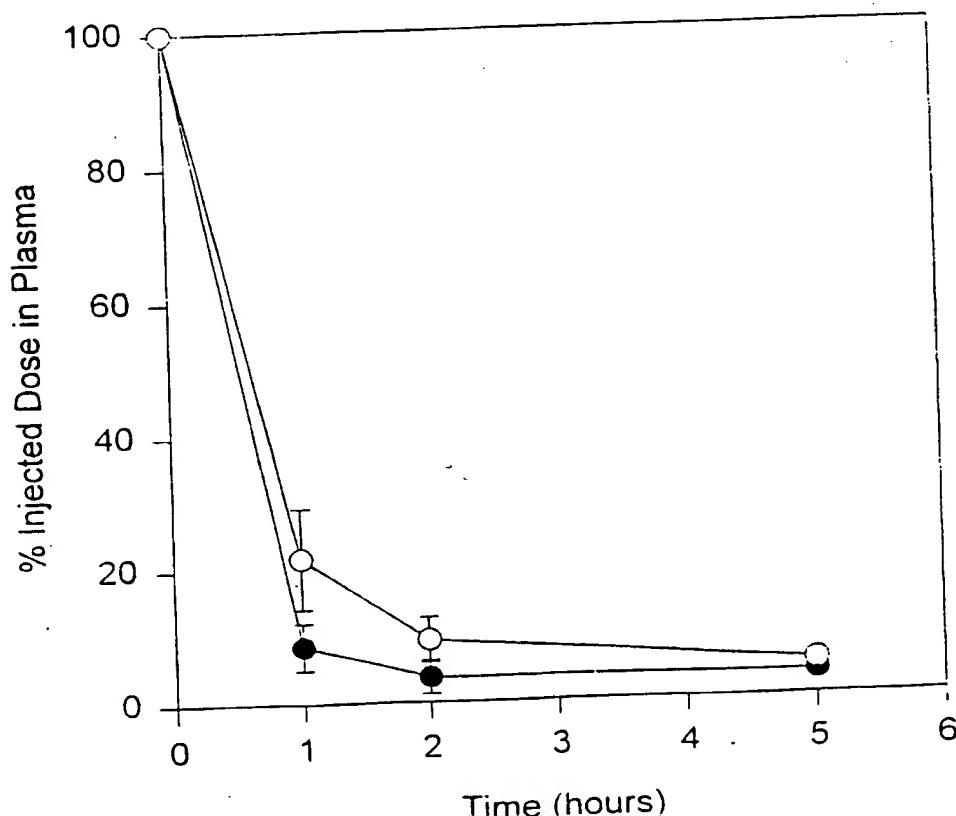


Figure 19B

05/31/96

02:42

604 264 9959

INEX

041/043

In Vivo Transfection in the Lung

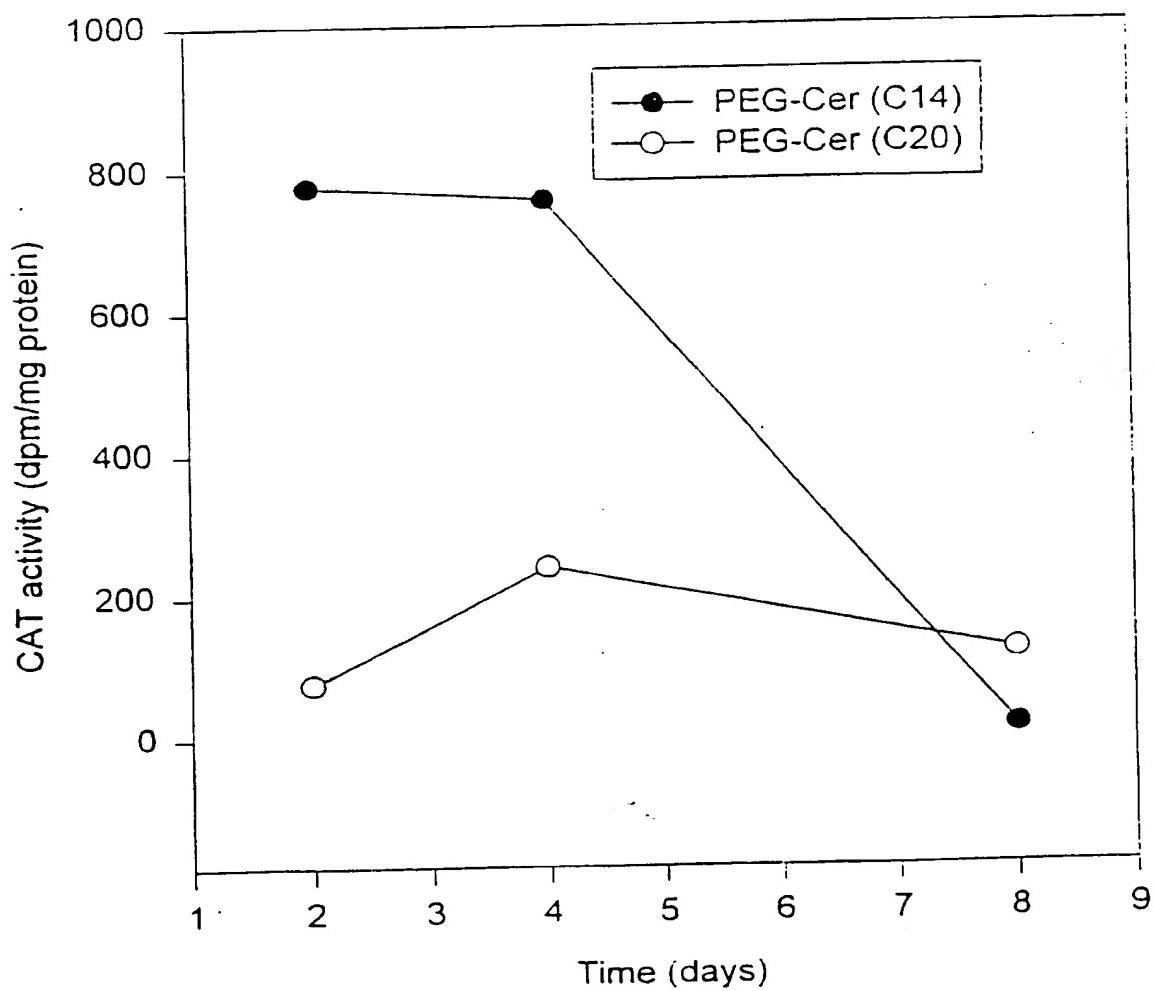


Figure 20

05/31/96 02:43 604 264 9959

INEX

042/043

In Vivo Transfection in the Liver

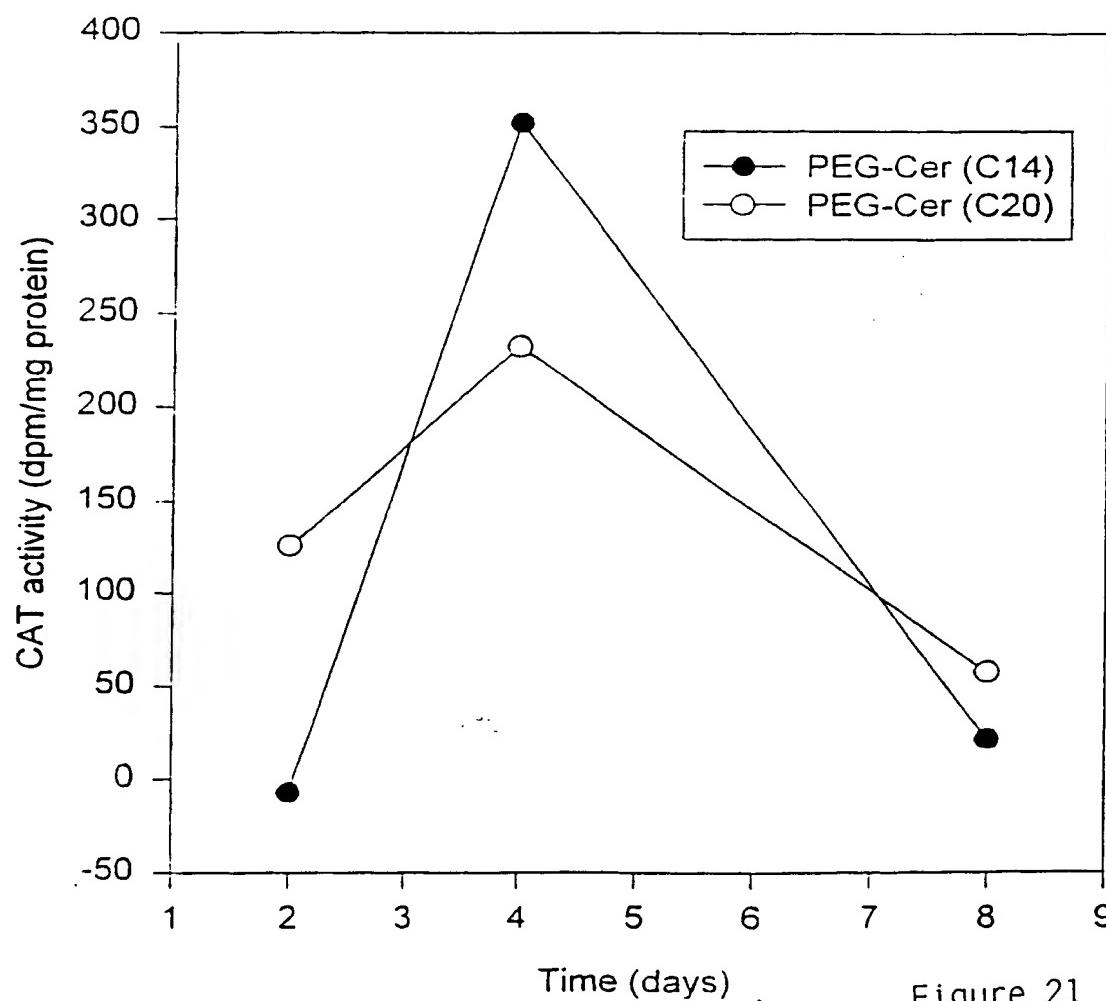


Figure 21

05/31/96 02:43

604 264 9959

INEX

043/043

In Vivo Transfection in the Spleen

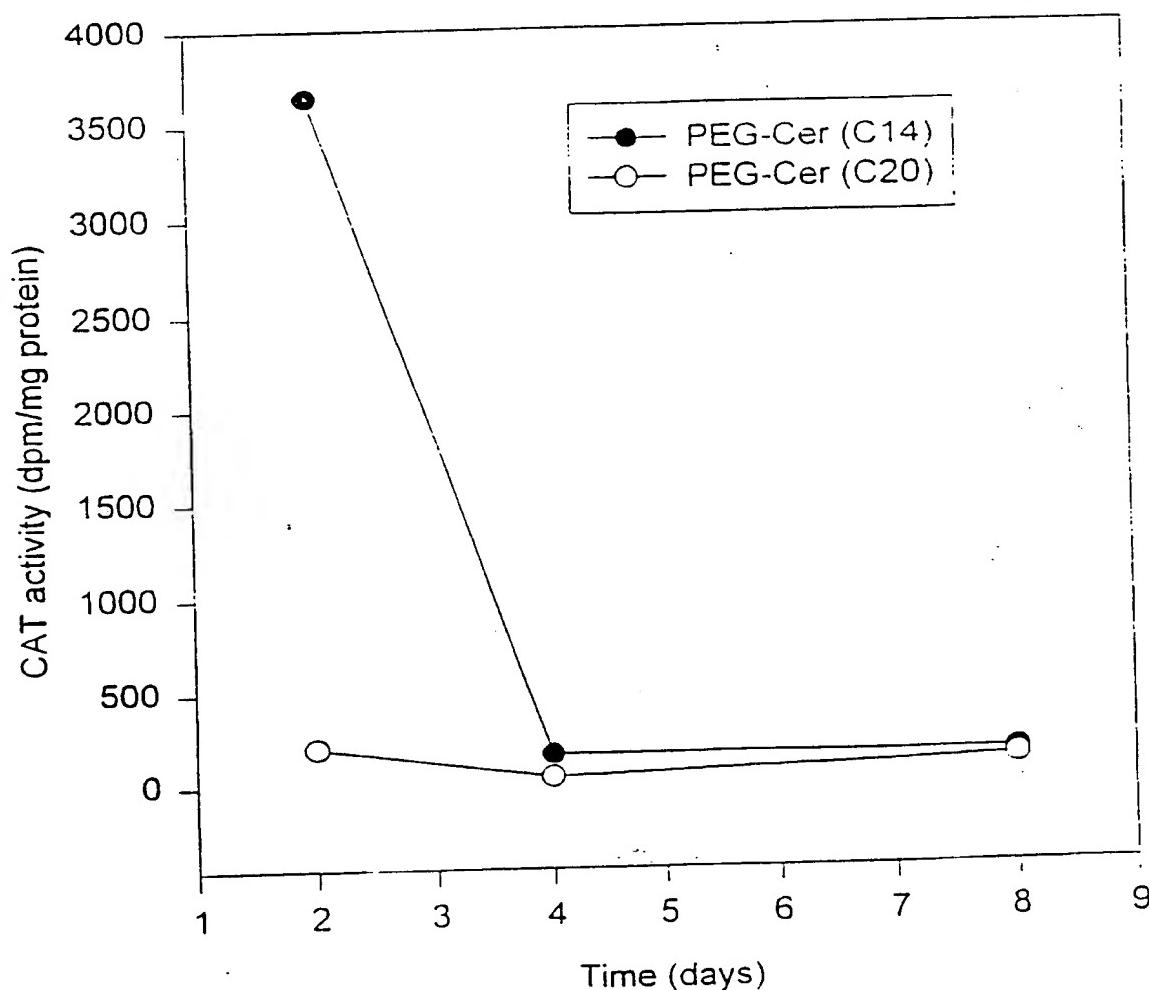


Figure 22

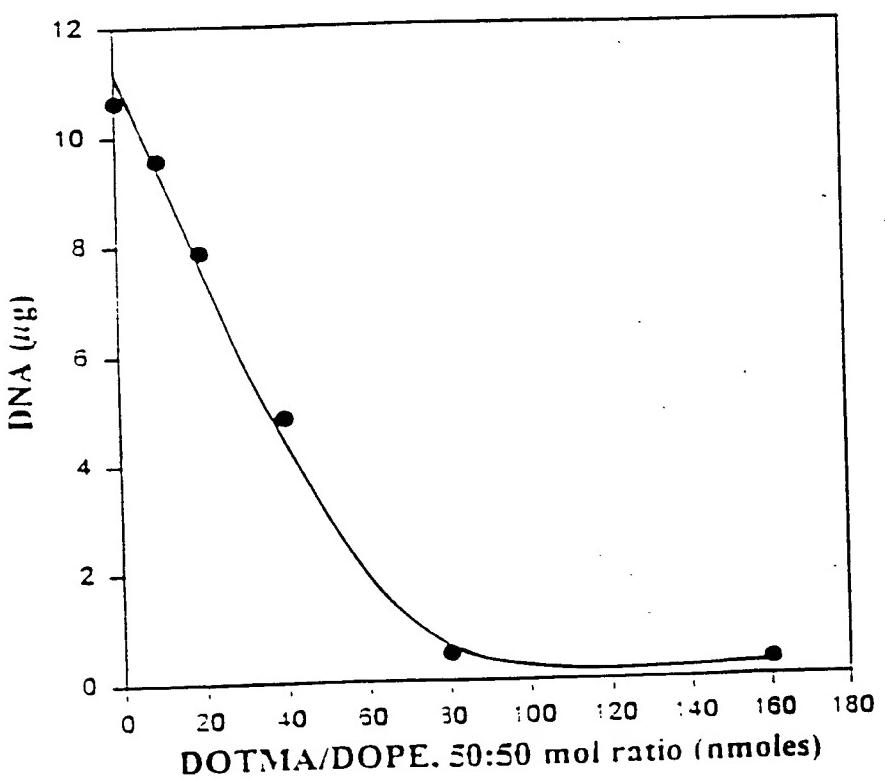


Figure 23

Figure 24A

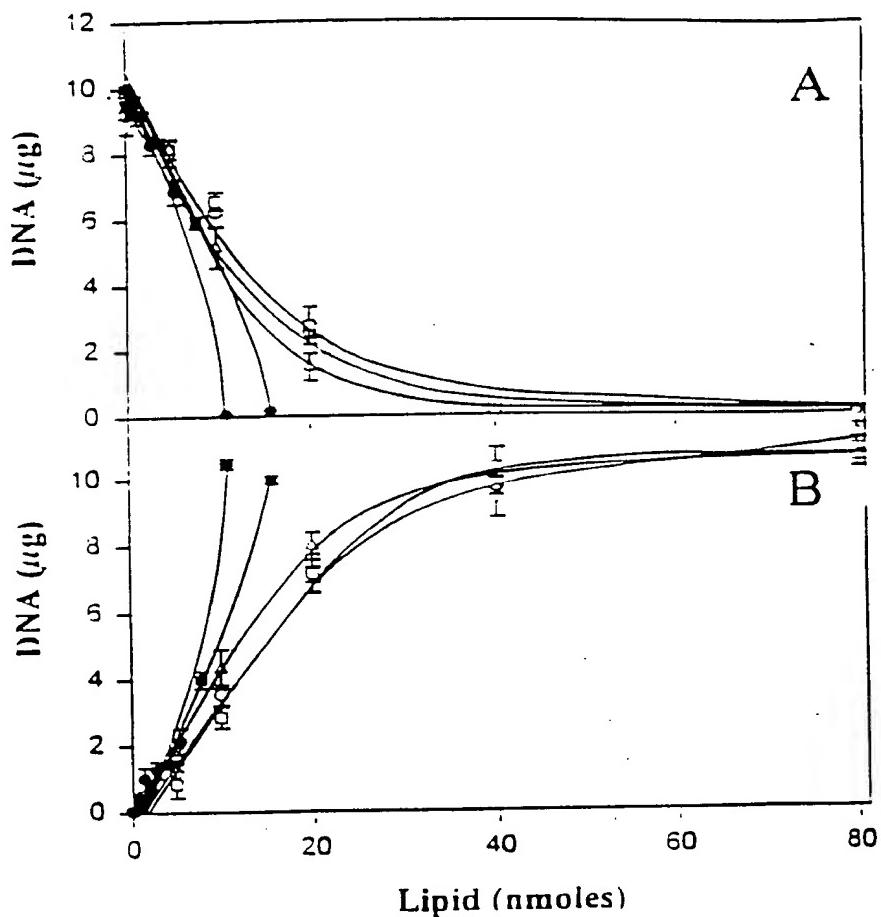
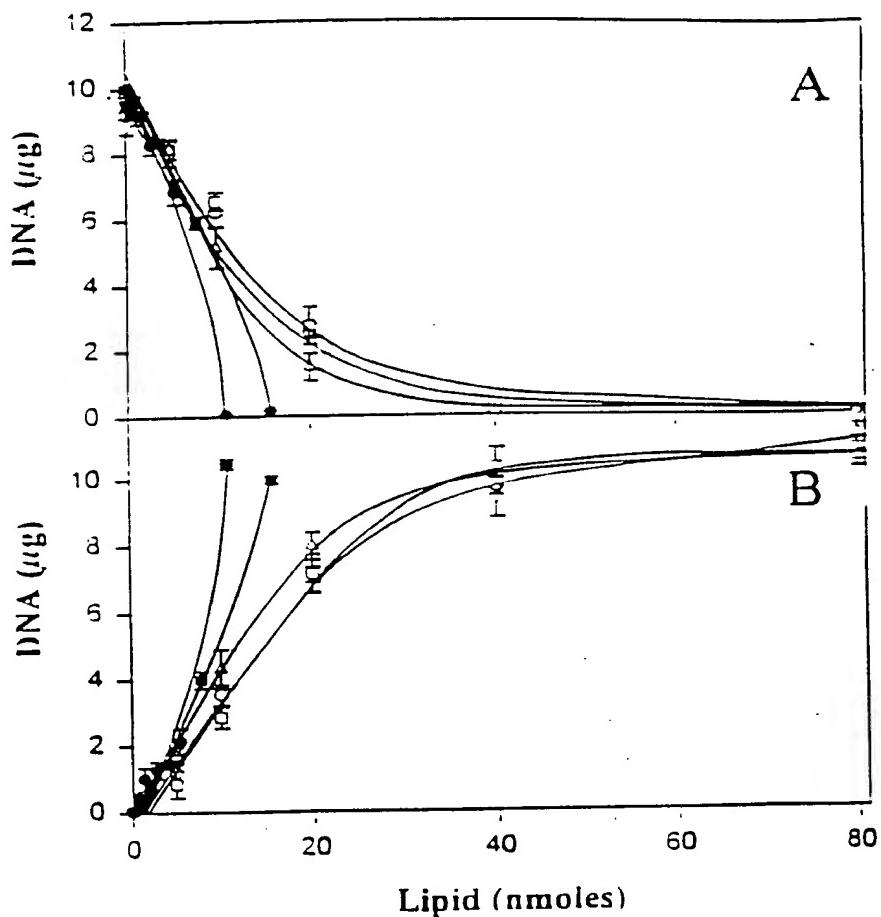


Figure 24B



Recovery of plasmid DNA in the aqueous (A) and solvent (B) phase following Bligh and Dyer extraction of the DNA/lipid complexes. DNA amount used was 10 μg. Monocationic lipids used were DDAB (O), DOTMA (□) and DODAC (Δ). Lipopolyamines used were Lipofectamine (●), and Transfectam (■). All data points are averaged from three replications and expressed ± SEM.

Figure 25A

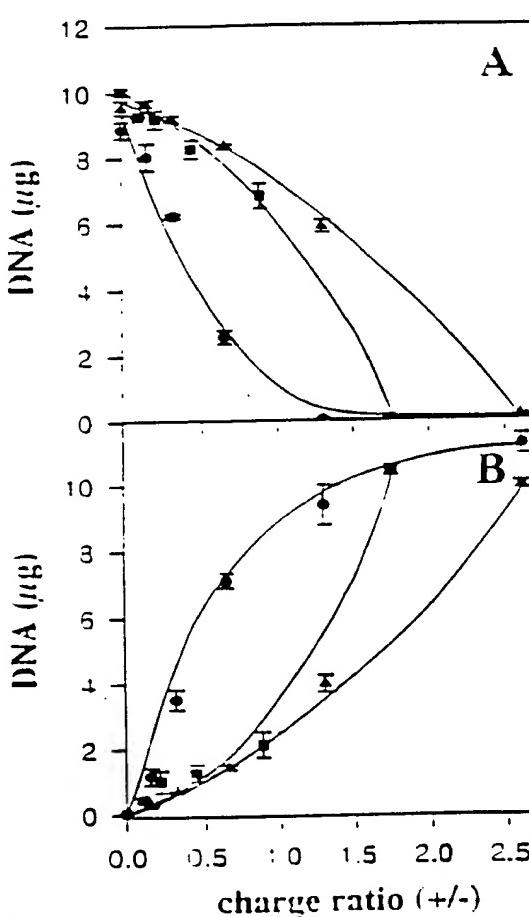


Figure 25C

Figure 25B

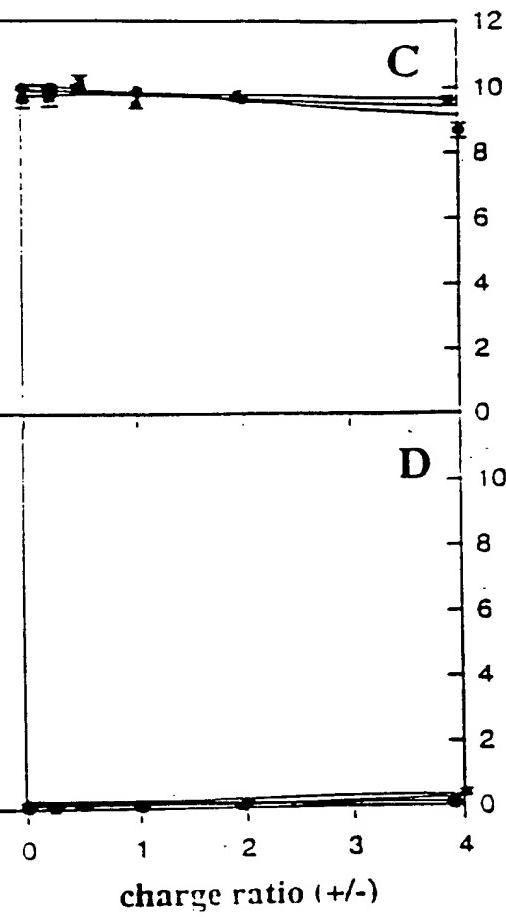


Figure 25D

REPRODUCED BY PERMISSION OF THE AUTHOR

The recovery of plasmid DNA from aqueous (A and C) and solvent (B and D) fractions following Bligh and Dyer extractions and expressed as a function of charge ratio (+/-). (A and B), DDAB (●), Lipofectamine (■) and Transfectam (▲). (C and D), the effects of other cations, calcium (●), L-lysine (■), and poly-L-lysine (▲). DNA amount used was 10 μ g and all data points were averaged from three experiments and presented \pm SEM.

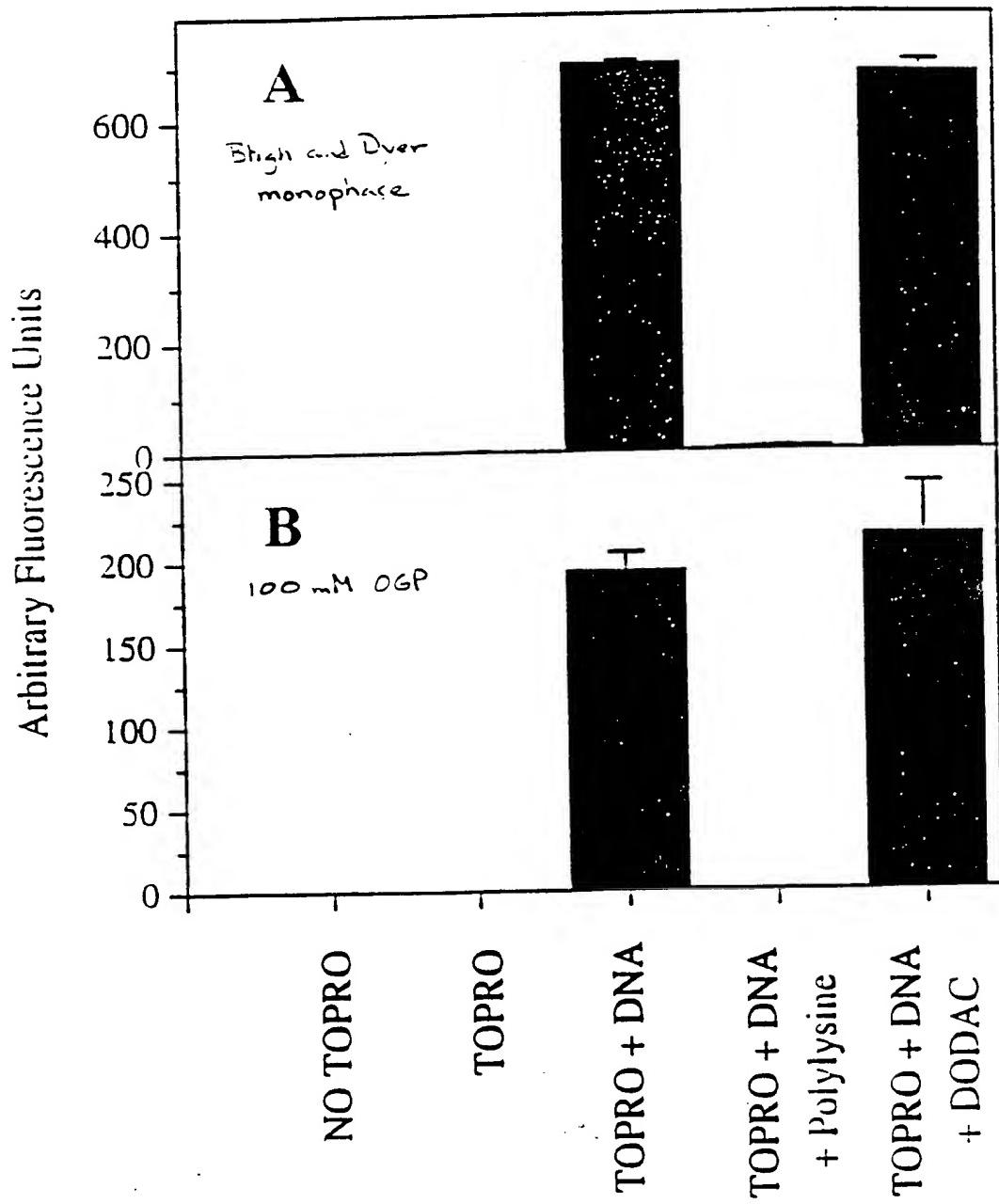
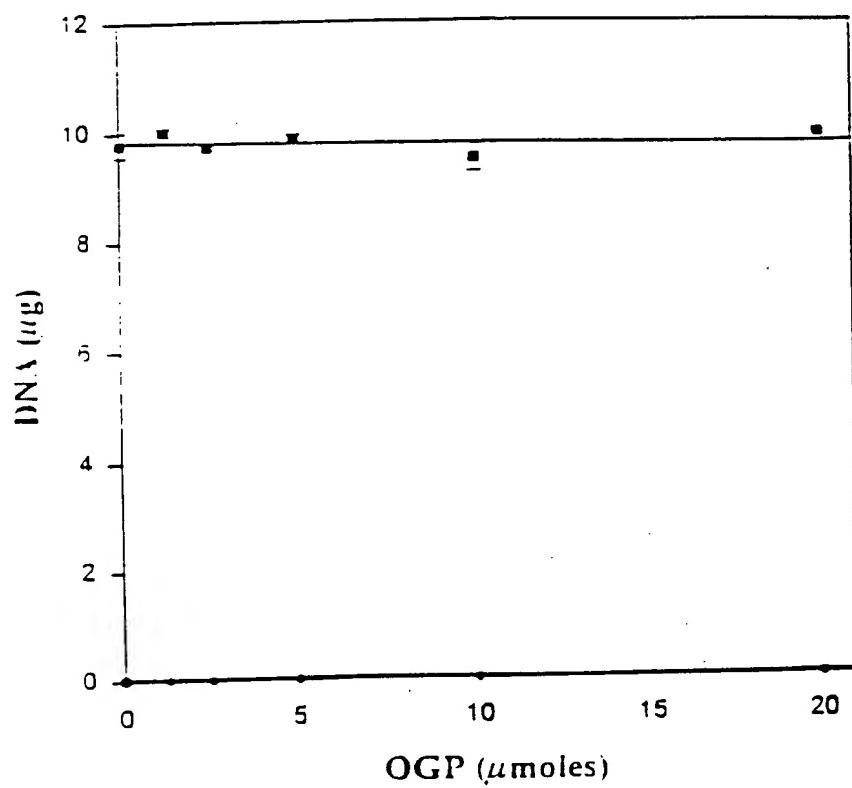


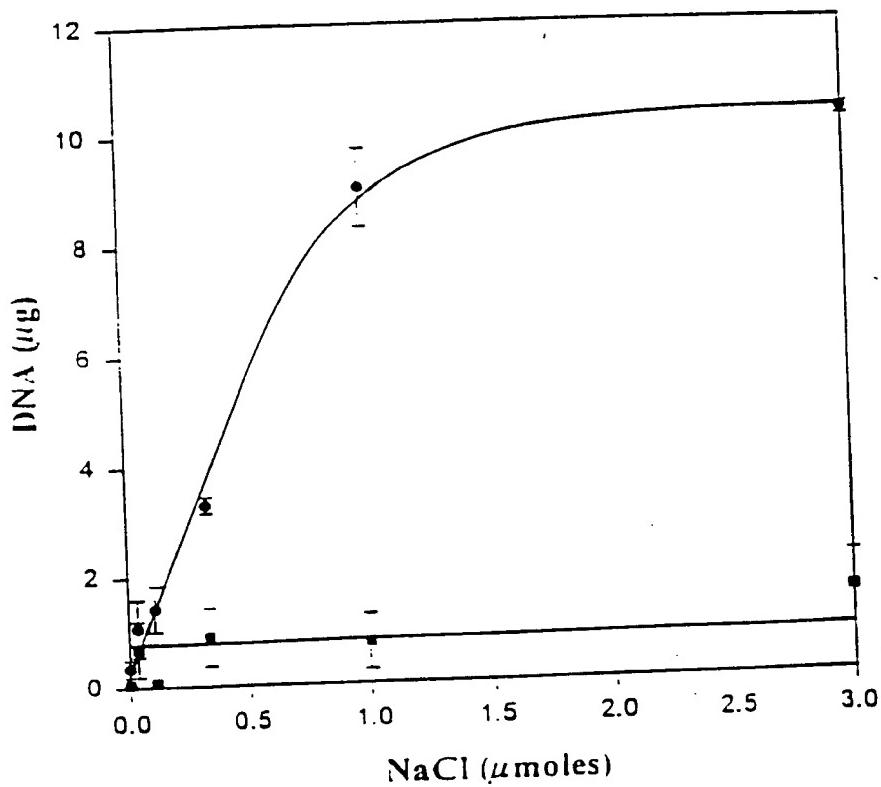
Figure 26A

Figure 26B



Effects of increasing amounts of OGP on the recovery of plasmid DNA from the aqueous (●) and solvent (■) phases following Bligh and Dyer extraction of

Figure 27



Effects of increasing amounts of NaCl on the recovery of plasmid DNA from the aqueous phase following Bligh and Dyer extraction of DNA/lipid complexes. Amount of DNA used was 10 μ g. DODAC (●), Lipofectamine (■).

Figure 28

A

8 7 6 5 4 3 2 1 m

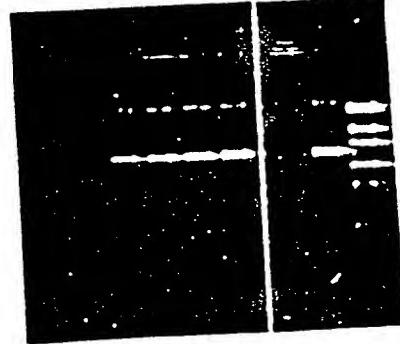


Figure 29A

4 3 2 1 m B

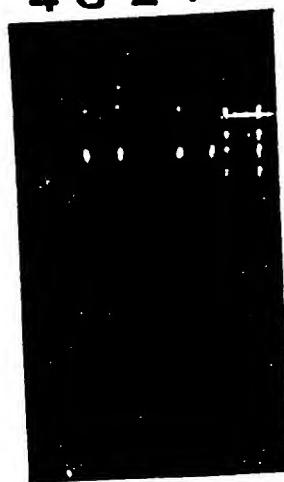


Figure 29B

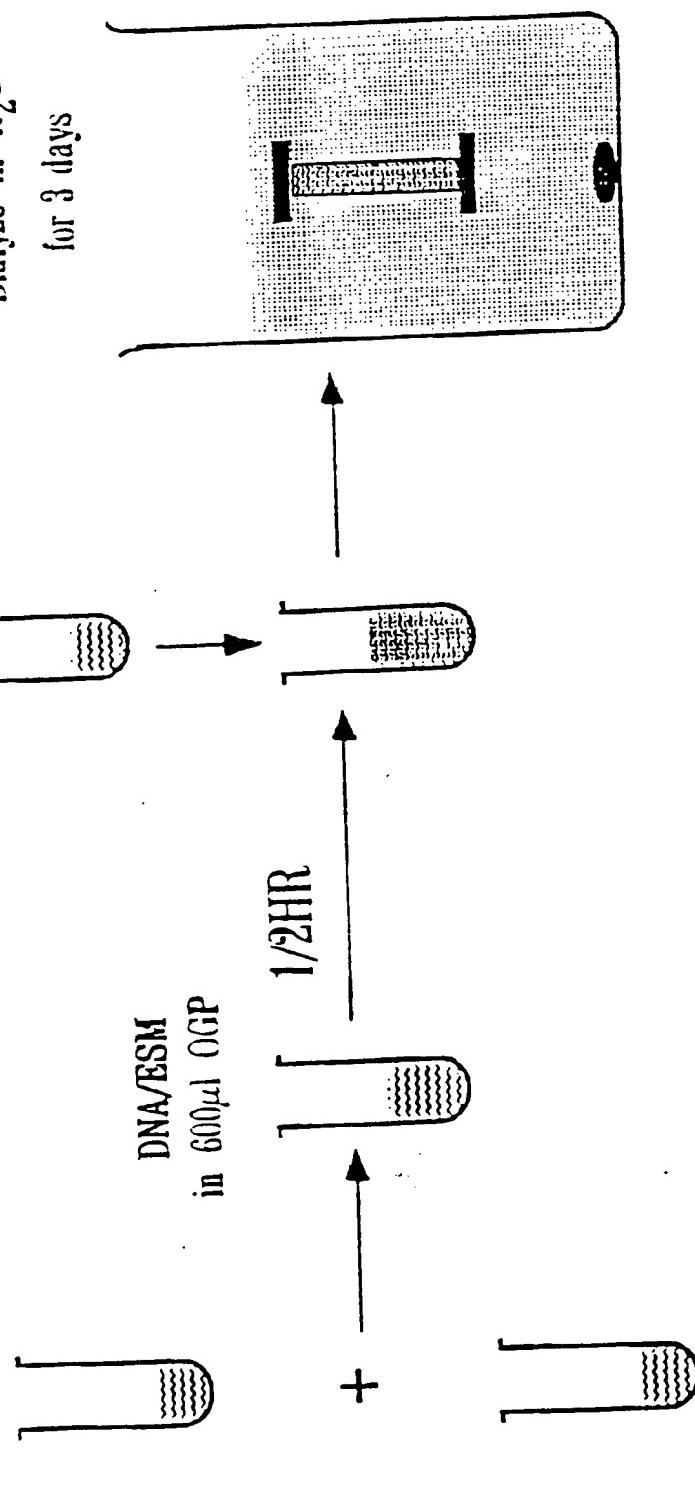
160nmoles DODAC

in 400 μ l OGP

SONICATED

10 μ g DNA
in 200 μ l OGP

Dialyze in H₂O
for 3 days



160nmoles ESM
in 400 μ l OGP
SONICATED

Figure 30

$20\mu\text{g}$ β -gal DNA/ 160nmoles DODAC/ 320nmoles ESM

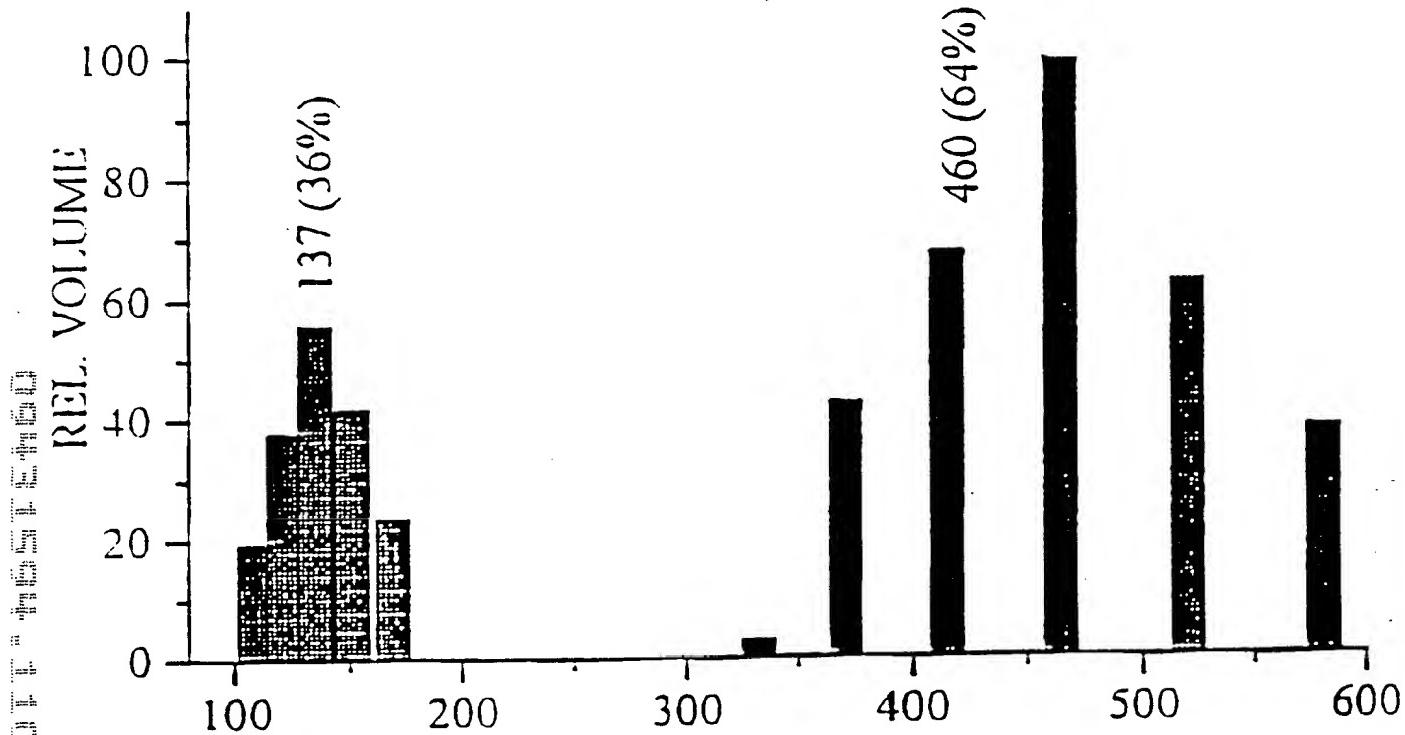


Figure 31A

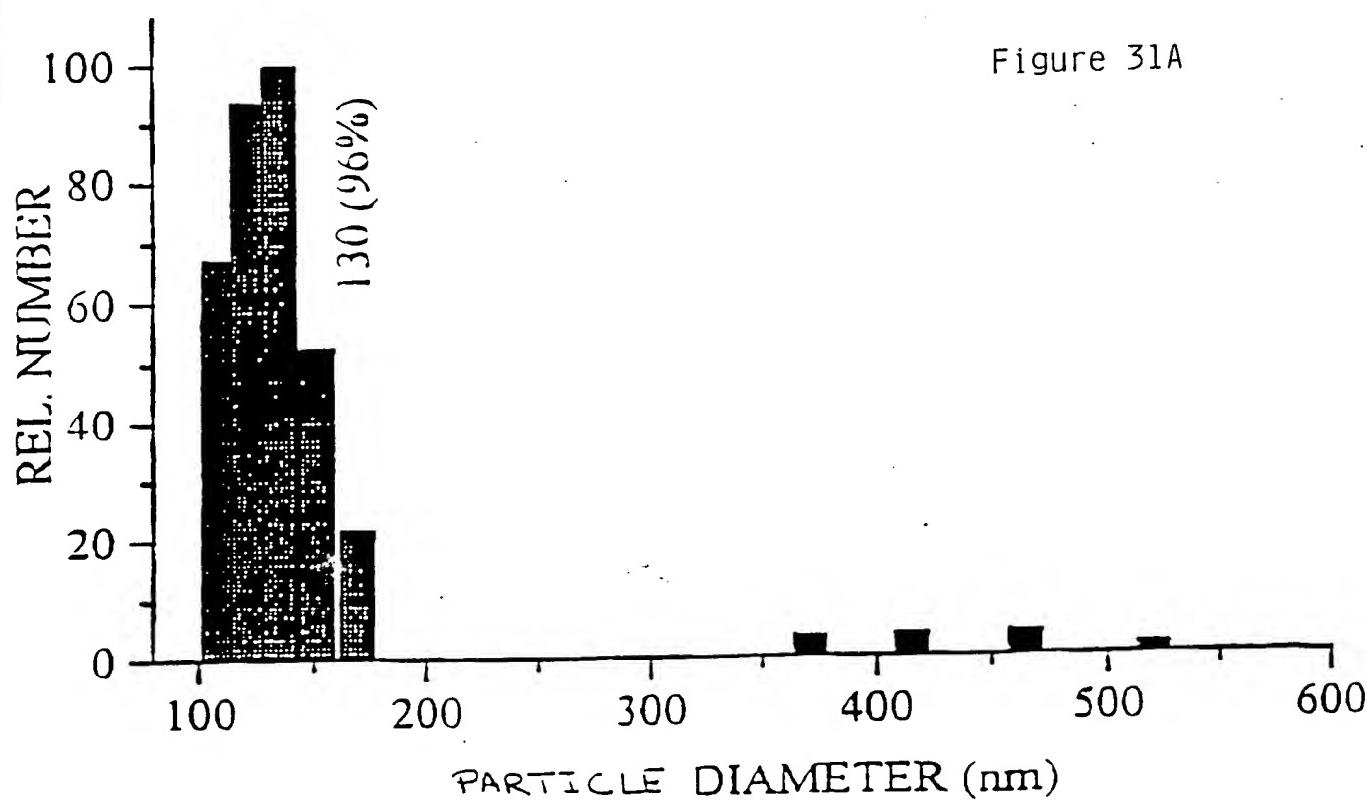
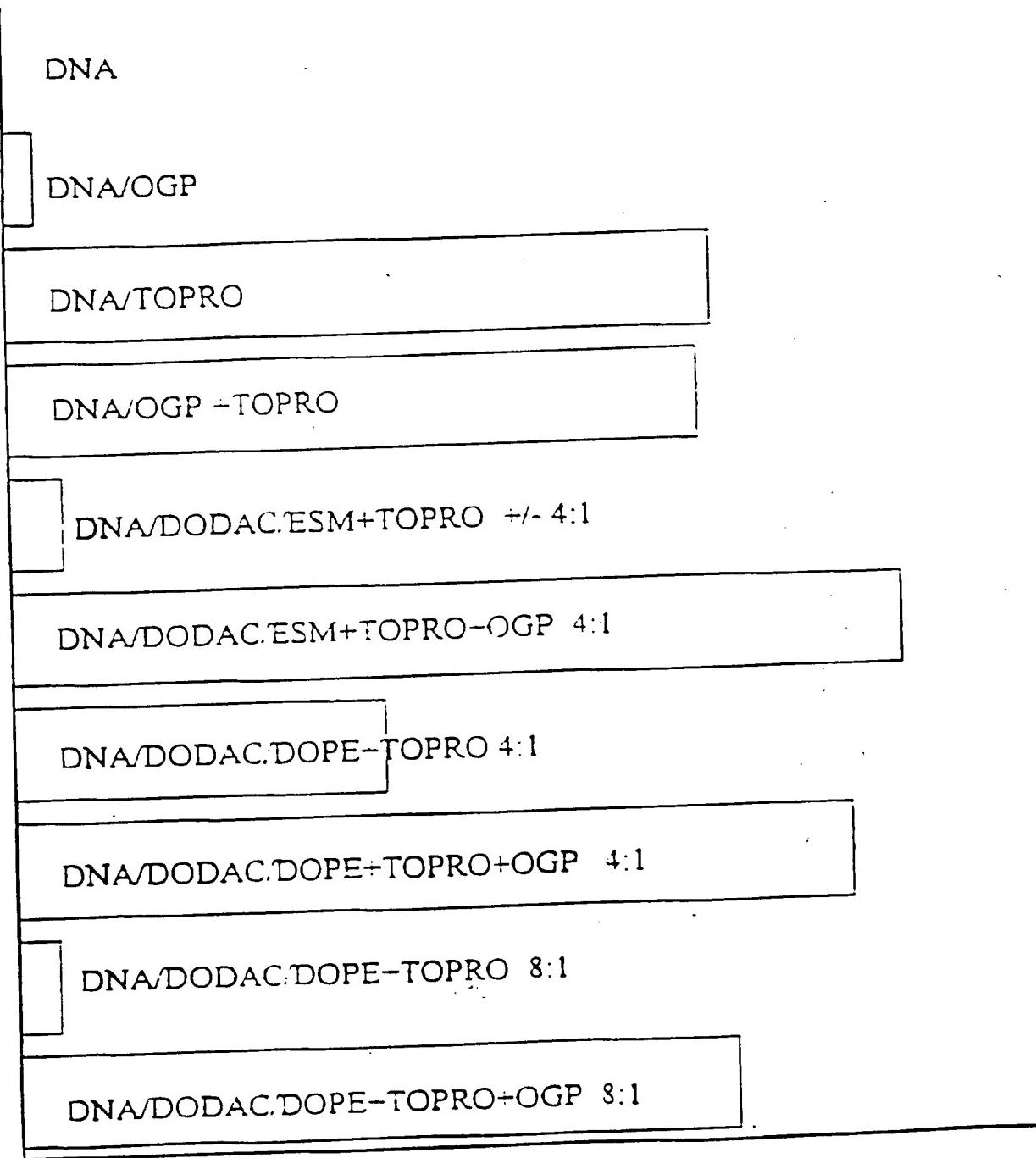


Figure 31B

09431534 - 1109199



FLUORESCENCE INTENSITY

Figure 32

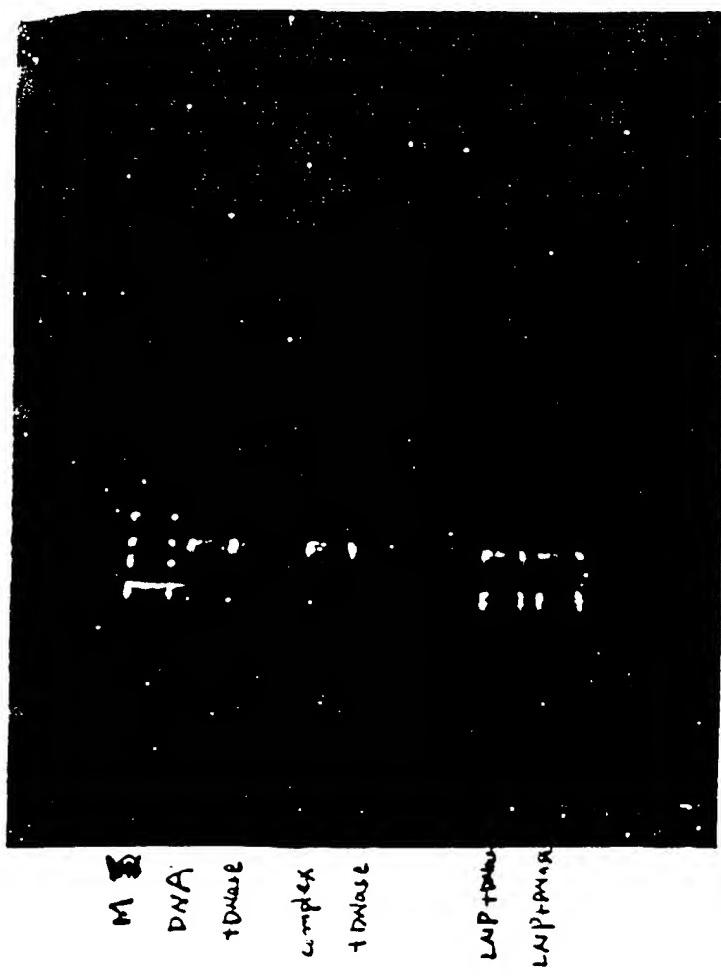
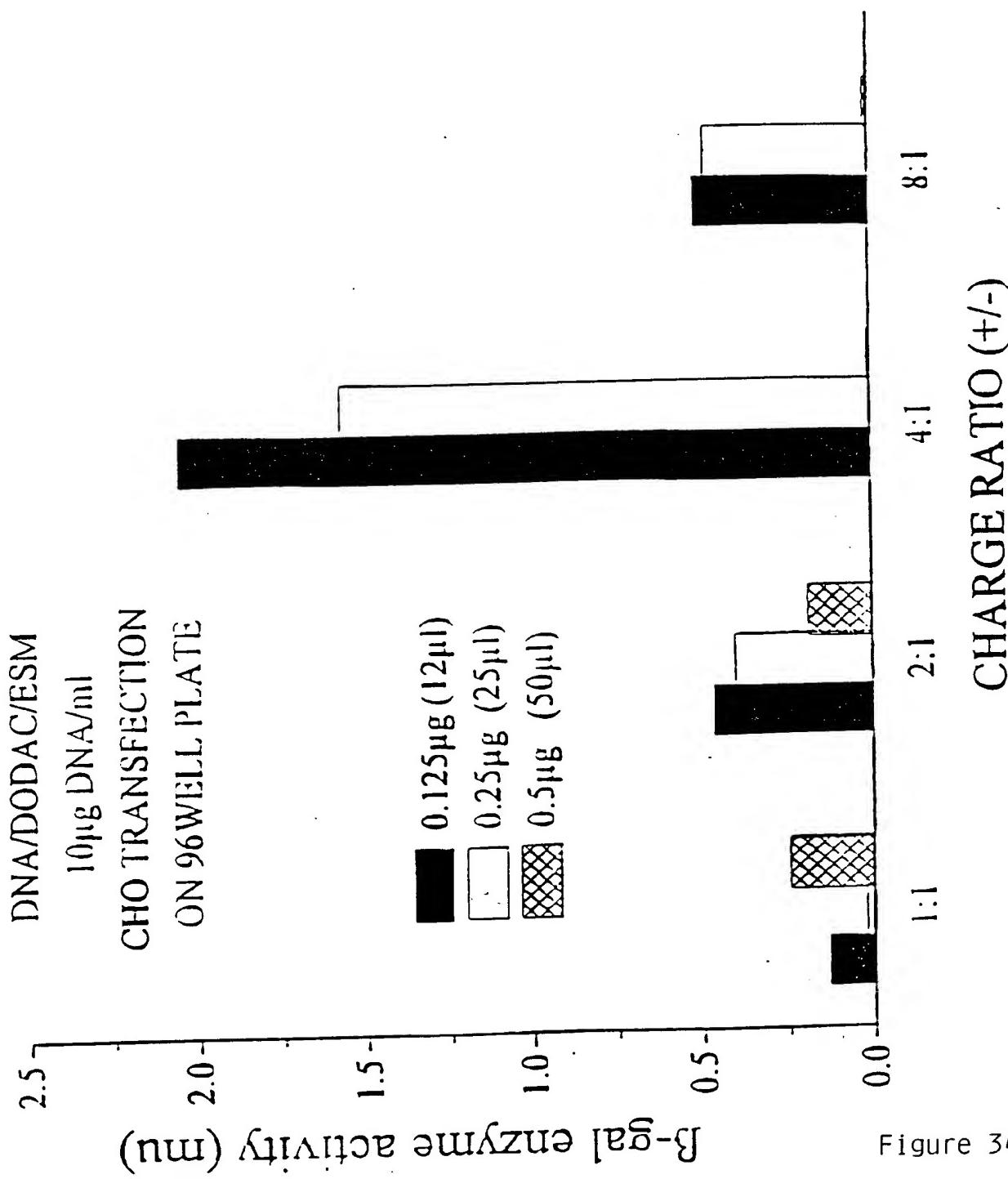


Figure 33



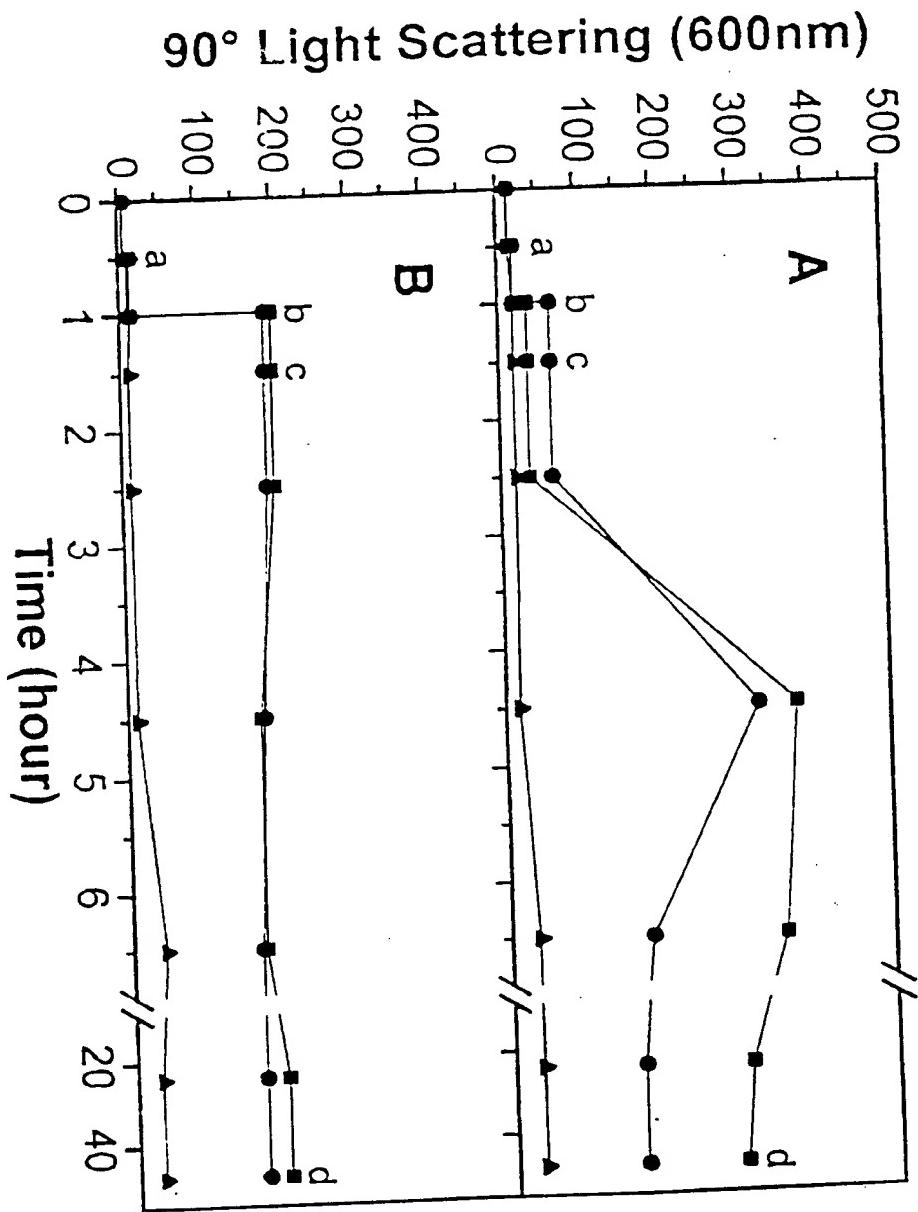


Figure 35A

Figure 35B

90° Light Scattering (600nm)

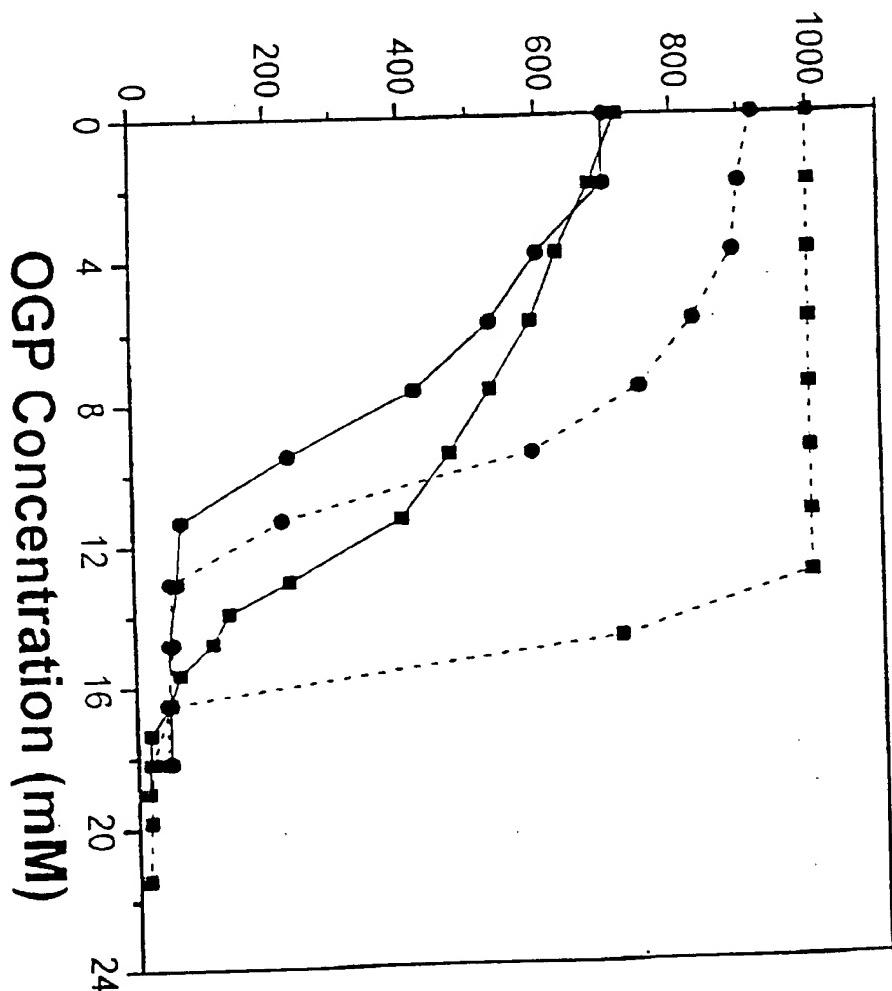


Figure 36

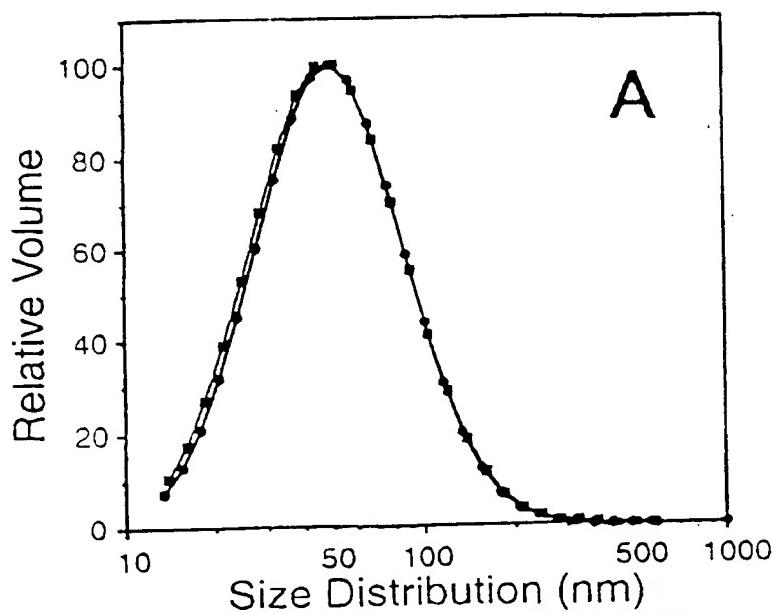


Figure 37A

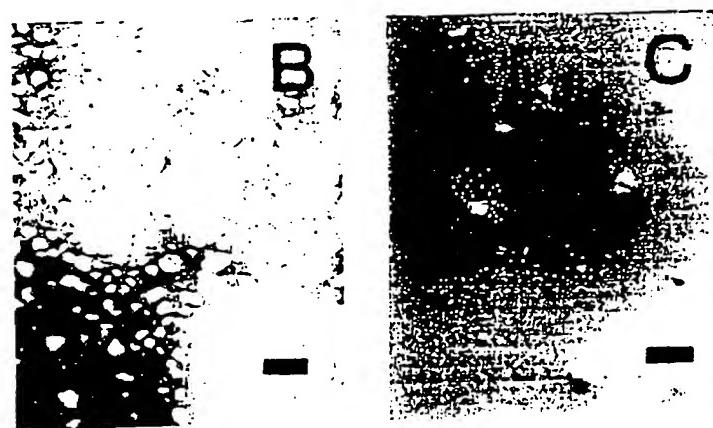


Figure 37B

Figure 37C

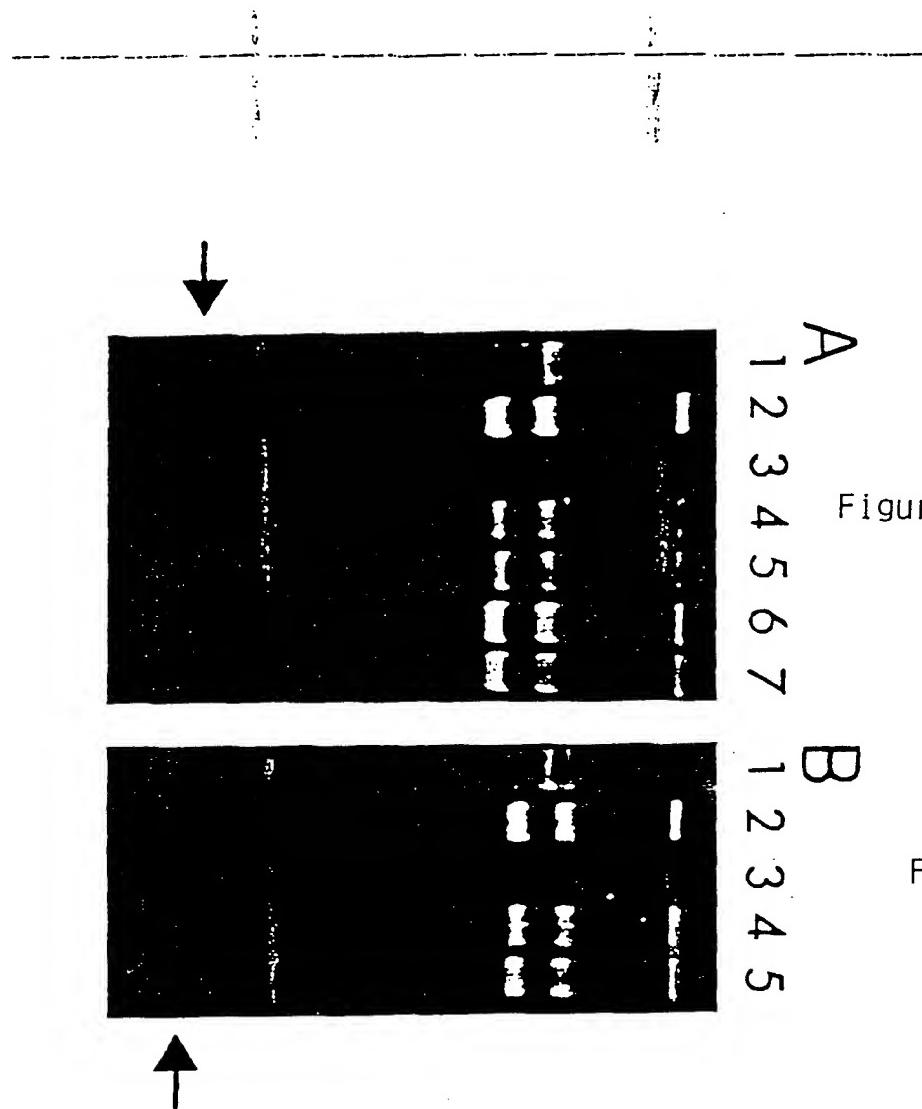


Figure 38A

Figure 38B

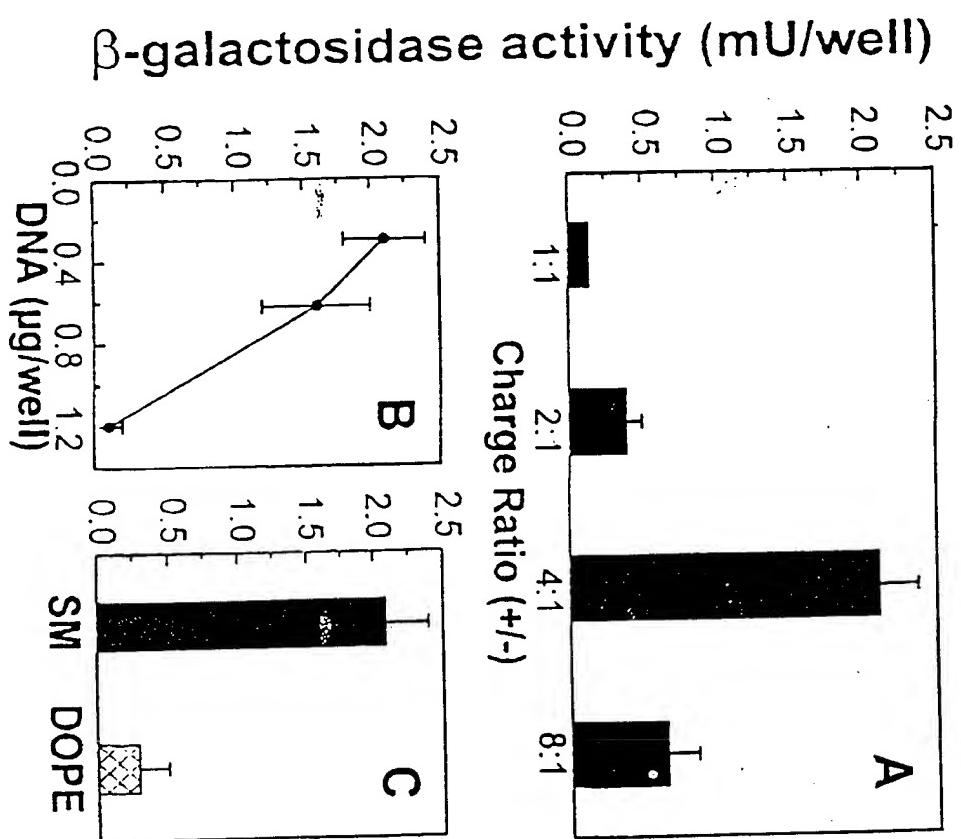
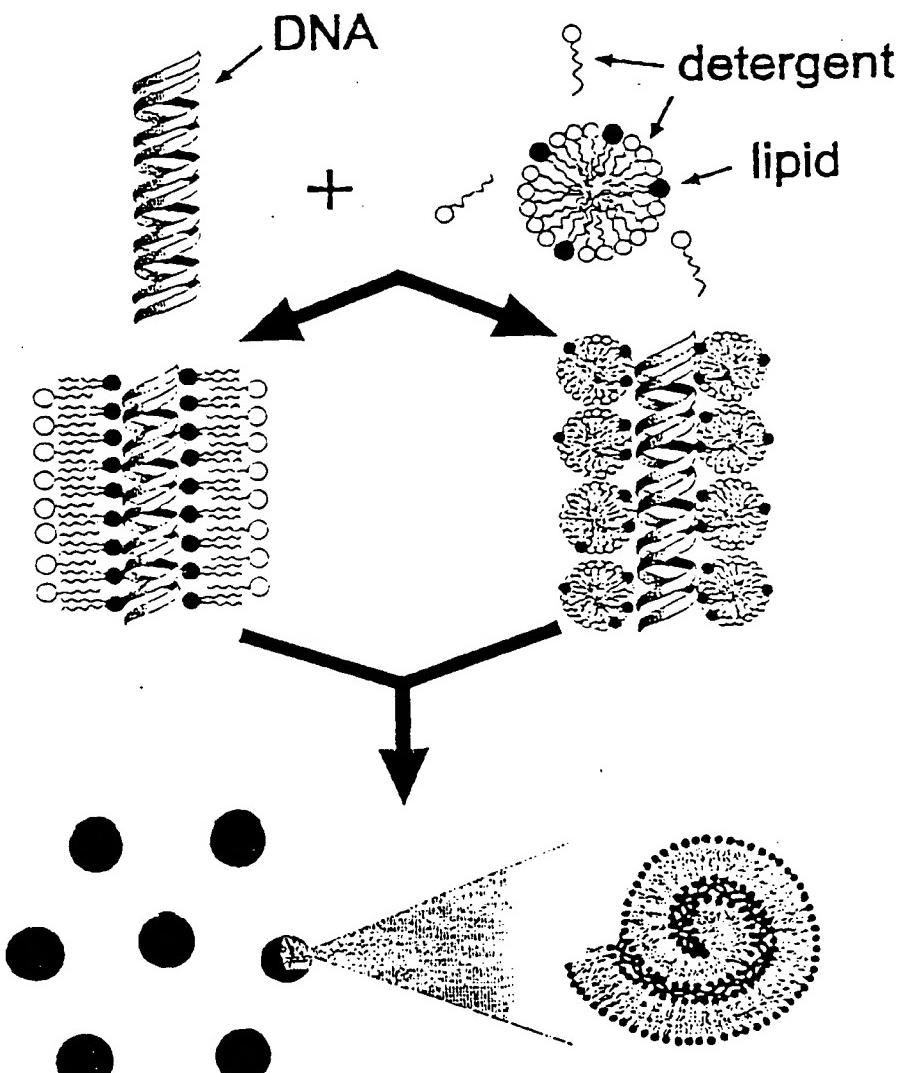


Figure 39B

Figure 39C

Figure 39A



DNA-Lipid Particle Formation

Figure 40

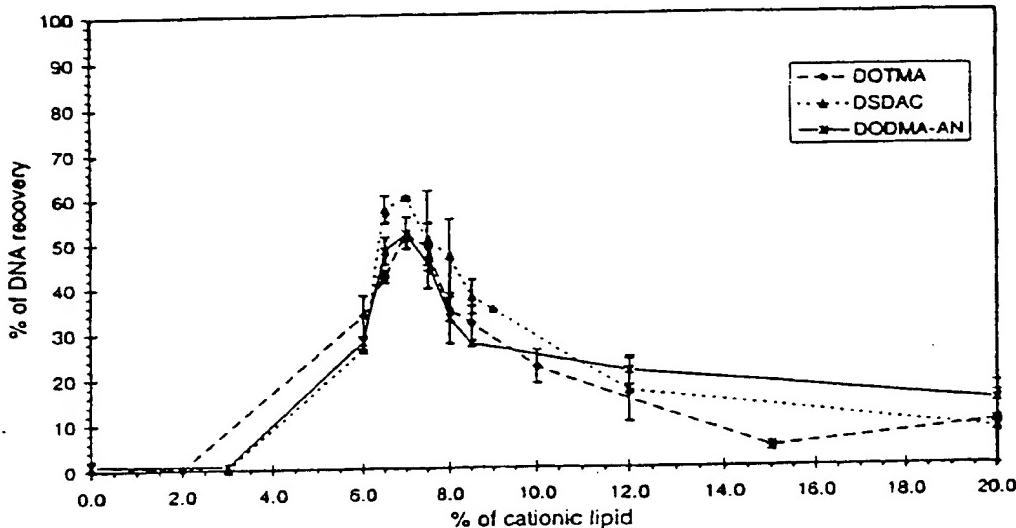
06/05/96 13:38

604 264 9959

INEX

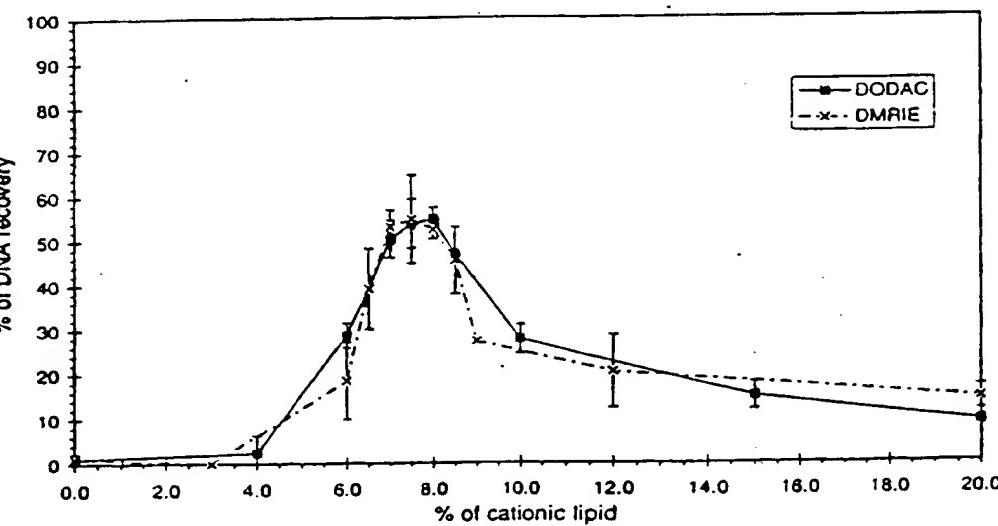
004/009
EXAMPLE 1

% Recovery of pNEXCAT with different composition of cationic lipid/DOPE/10 mol % PEG-CER C14 (5.0 umol total lipid) from DEAE Sepharose CL6B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)



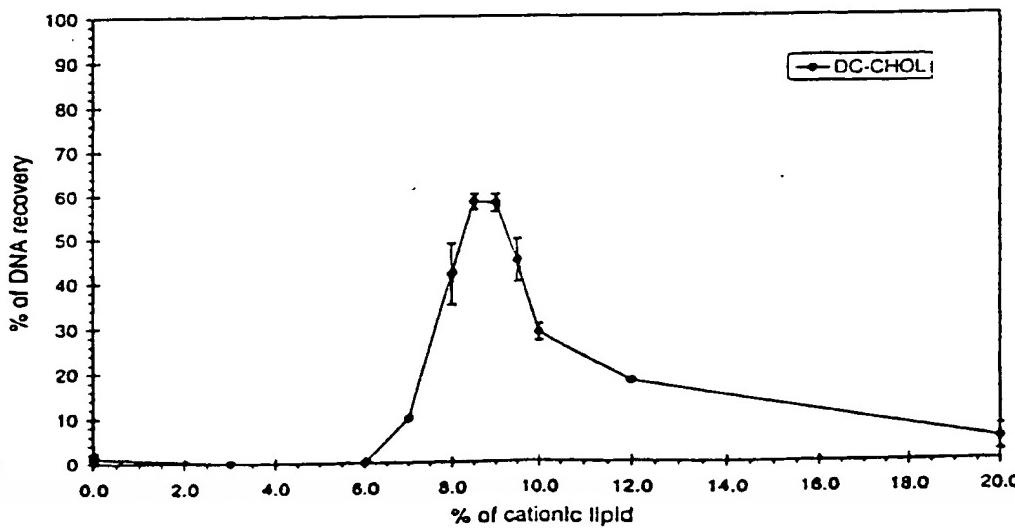
(a)

Figure 41A



(b)

Figure 41B



(c)

Figure 41C

06/05/96 13:39 604 264 9959

INEX

005/009
EXAMPLE B

**Elution profile of 7.0 mol % DODAC/ 83 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)**

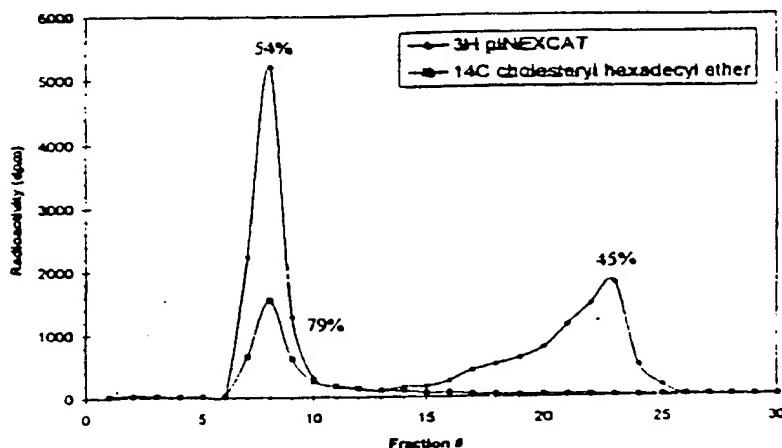


Figure 42A

(a)

**Elution profile of 7.0 mol % DOTMA/ 83 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)**

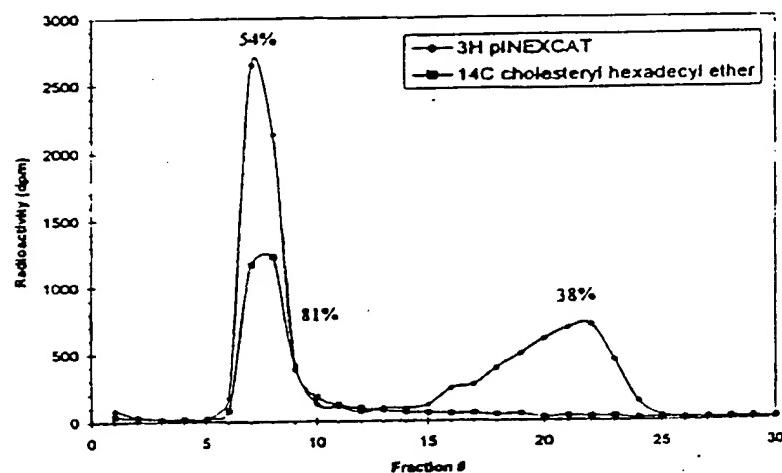


Figure 42B

(b)

**Elution profile of 7.5 mol % DSDAC/ 82.5 mol % DOPE/ 10 mol % PEG-CER C14
from Sepharose CL4B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)**

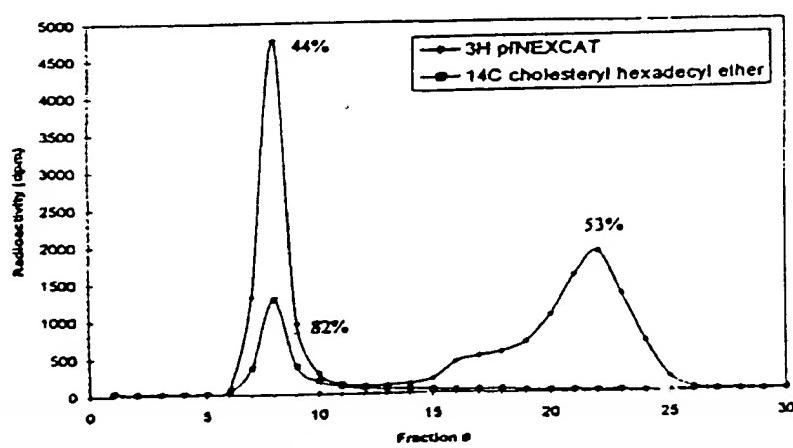


Figure 42C

(c)

06/05/96 13:40 604 264 9959
06/05/96 WED 07:51 FAX 604 822 4843

INEX
LIPOSOME RES UNIT

006/009
002

Cationic Lipid Titration of 50 μ g pCMV β in POPC:DOPE:PEG-CerC8:AL-1
(65-x:25:10:x) Liposomes As Analyzed by the PicoGreen Assay
Encapsulation Performed at pH4.8 or pH7.5

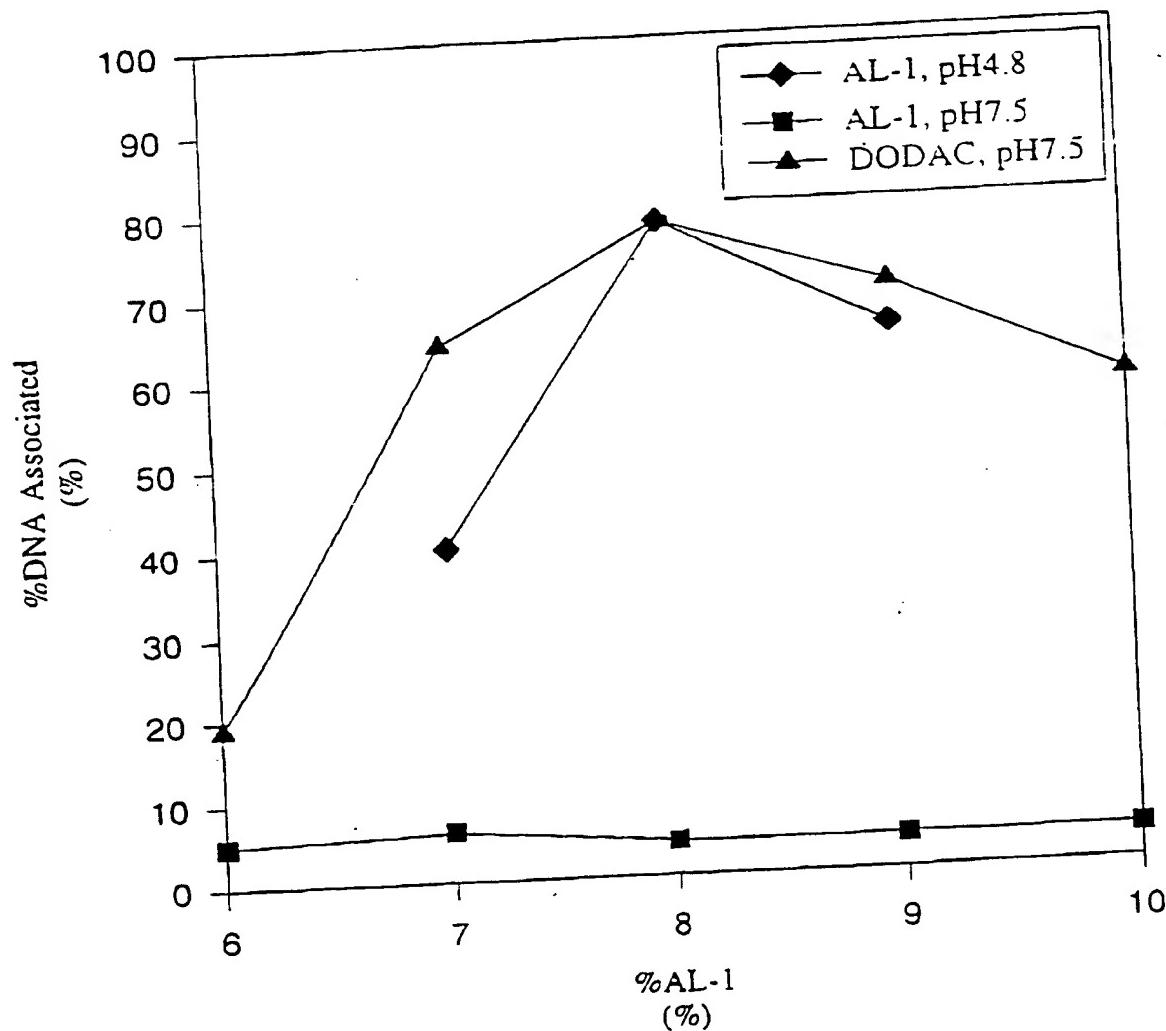


Figure 43

06/05/96 13:40 604 264 9959
06/04/96 TUE 16:08 FAX 604 822 4843

INEX
LIPOSOME RES UNIT

007/009
003

EXAMPLE D
(a)

Serum Stability (1.5 hr at 37°C) of 50 μ g pCMV β
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)
Liposomes {pH4.8 Encapsulation}

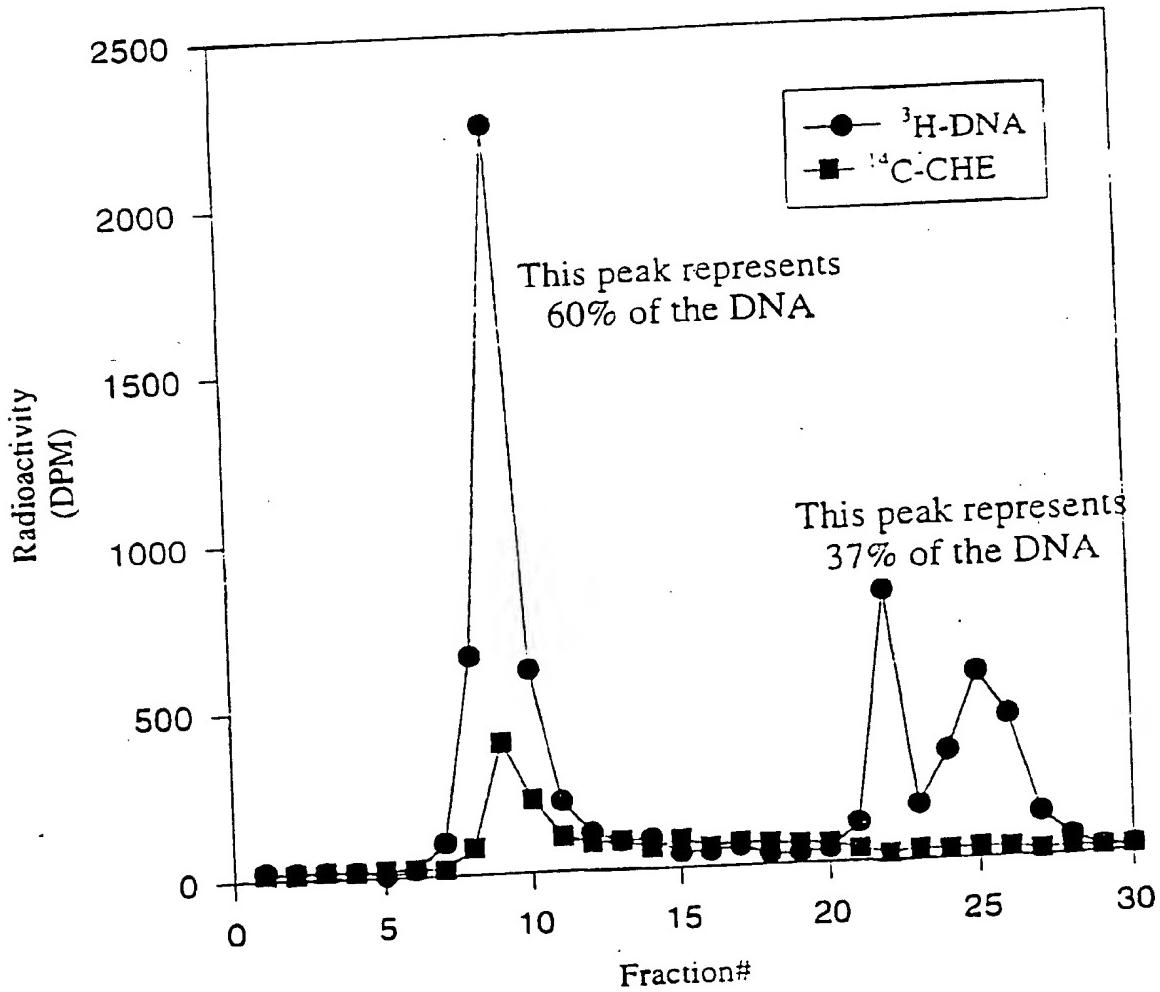


Figure 44

06/05/96 13:41 604 264 9959
06/04/96 TUE 16:08 FAX 604 822 4843

INEX
LIPOSOM RES UNIT

008/009
004

EXAMPLE D

(b)

Serum Stability (1.5 hr at 37°C) of 50μg pCMV β
Encapsulated in POPC:DOPE:PEG-CerC8:AL-1 (57:25:10:8)
Liposomes {pH7.5 Encapsulation}

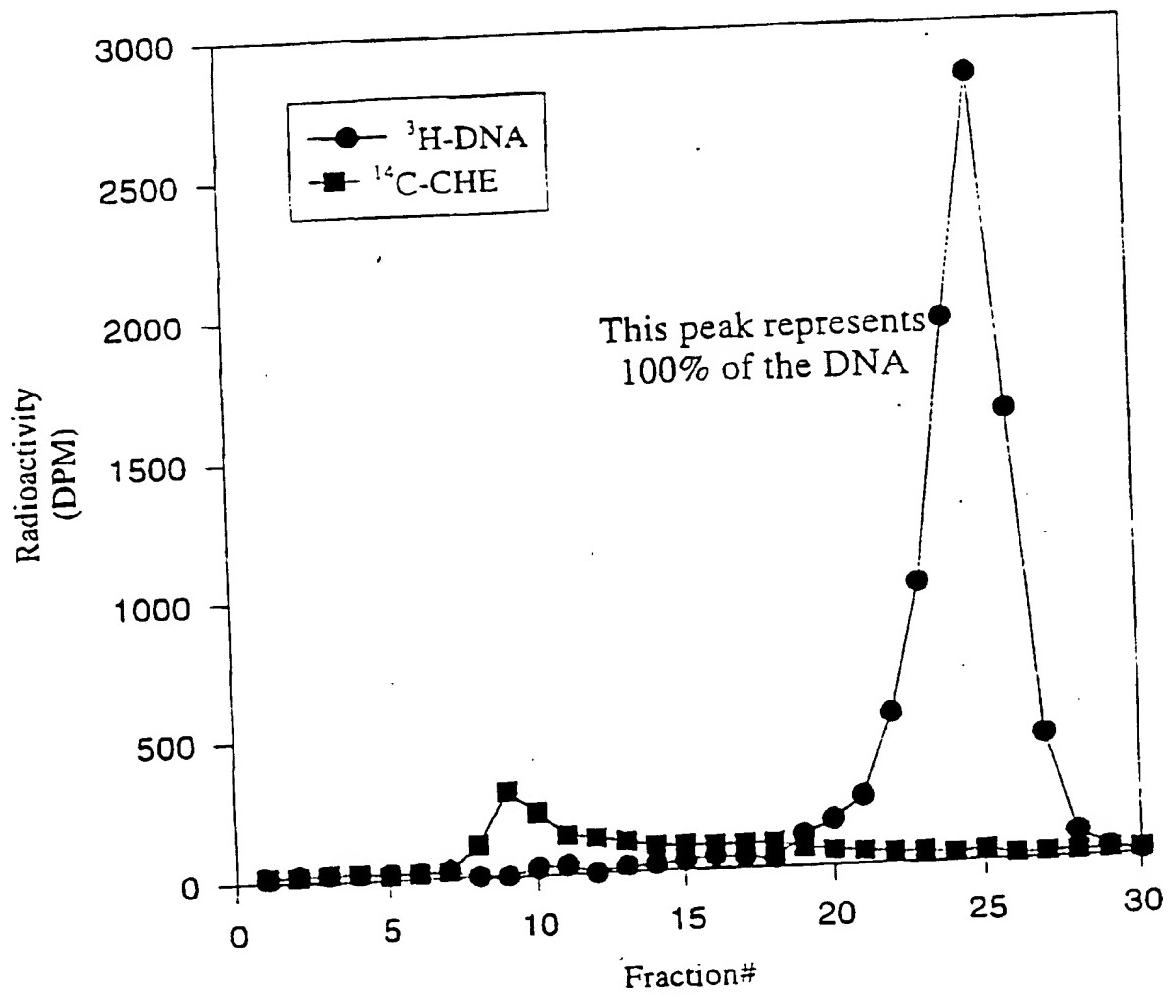


Figure 45

06/05/96 13:41 604 264 9959

INEX

009/009

EXAMPLE E

Effect of PEG-CER C14 in 7.5 mol % DODAC/DOPE/PEG-CER C14 system (5.0 umol lipid) on the recovery of 3H pINEXCAT and 14C cholesteryl hexadecyl ether from DEAE Sepharose CL6B column in 150 mM NaCl, 20 mM HEPES (pH 7.4)

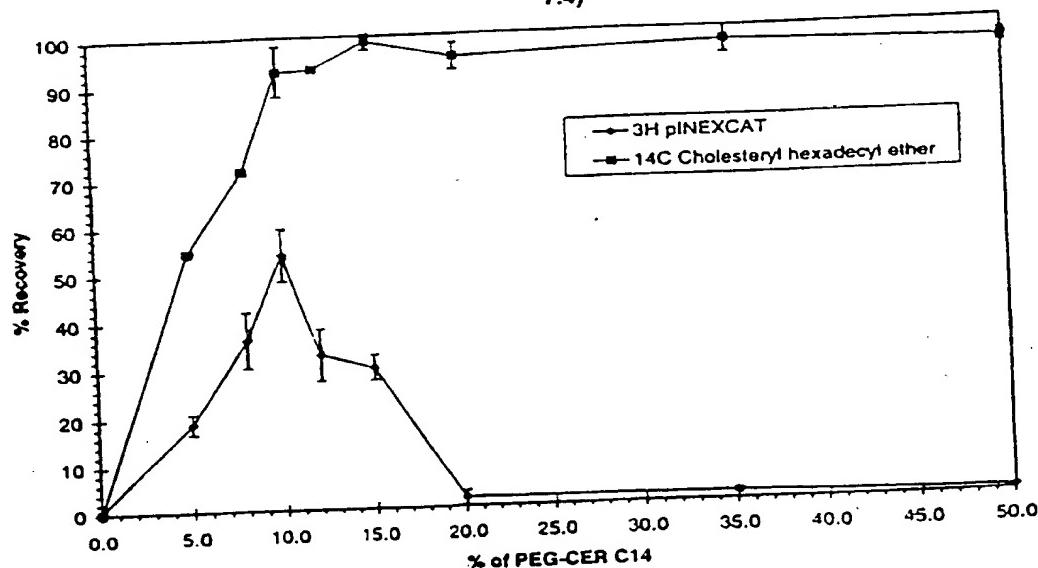


Figure 46